CHRONIC TOXICITY SUMMARY

ETHYLENE

(Ethene; acetene; bicarburetted hydrogen; olefiant gas; elayl)

CAS Registry Number: 74-85-1

I. Chronic Toxicity Summary

Inhalation reference exposure level	**100 μg/m^3**
Critical effect(s)	Decreased hematocrit, hemoglobin and neutrophils, and decreased lymphocytes in humans. Based on toxicity of the metabolite, ethylene oxide.
Hazard index target(s)	Circulatory system; immune system

II. Chemical Property Summary (HSDB, 1995)

Molecular formula	C_2H_4
Molecular weight	28.12 g/mol
Description	Colorless gas; olefinic odor; slightly sweet
Vapor pressure	4270 kPa at 0°C
Solubility	Very slightly soluble in water (131 mg/L H_2O at 20°C). Slightly soluble in acetone, benzene and ethanol. Soluble in diethyl ether
Conversion factor	1.15 μg/m^3 per ppb at 25°C

III. Major Uses and Sources

Ethylene is a petrochemical produced in large quantities worldwide and is ranked 4th in weight made among organic chemicals produced in the U.S. (C&EN, 1995). Over 95% of worldwide annual commercial production of ethylene is currently based on steam cracking of petroleum hydrocarbons. In the U.S. ethane is the primary feedstock to produce ethylene. Commercially produced ethylene is then used as a feedstock for production of polymers and industrial chemicals. A small amount is used for controlled ripening of citrus fruits, tomatoes, bananas and other fruits, vegetables and flowers. Ethylene is ubiquitous in the environment, from both natural and man-made sources. It is a natural product of vegetation of all types and acts as an endogenous plant growth regulator. Endogenous but unidentified sources of ethylene exist in man and animals. A major anthropogenic source is burning vegetation. Ethylene is also released from agricultural wastes and refuse and from the incomplete combustion of fossil fuels. Small amounts are found in volcanic emissions and natural gas. There is little chance of inhalation

exposure during its manufacture because the process takes place in a closed system. Exposure may result from spills, leaks or use of ethylene.

Ethylene concentrations of <1-5 $\mu g/m^3$ occur in rural and remote sites while concentrations of 2 to over 1000 $\mu g/m^3$ can occur in urban and indoor sites (IARC, 1994). High indoor concentrations generally depend on whether burning biomass is used as a source of energy. Exposure to ethylene is 10 times greater in cigarette smokers than the exposure in polluted urban air (Persson *et al.*, 1988).

IV. Effects of Human Exposure

There is a lack of toxicological data on long-term ethylene exposure in humans. Inhalation pharmacokinetics of ethylene have been performed in human volunteers (Filser *et al.*, 1992). Due to ethylenes' poor solubility in blood, the accumulation factor "body/air" at steady-state was determined to be only 0.33$\pm$0.13 (mean$\pm$SD). The rate of metabolism was directly proportional to the exposure concentration (1^{st} order kinetics) in the range between 1 ppm and 50 ppm. The authors assume that at higher concentrations saturation of human ethylene metabolism occurs, similar to observations in rats (Bolt and Filser, 1987). Only 2% of ethylene inhaled was metabolized to ethylene oxide, whereas 98% of ethylene was exhaled unchanged. (Ethylene oxide is known to be carcinogenic in rodents.) The half-life of ethylene was determined to be 0.65 hr. The researchers also determined that the endogenous production of ethylene in the human subjects was 32 nmol/hr.

The measurement of hydroxyethyl adducts to N-terminal valine in hemoglobin has been used as dosimetry for ethylene oxide in occupational studies of fruit store workers exposed to ethylene (Törnqvist *et al.*, 1989). With an average exposure of 0.3 ppm of ethylene, it was estimated from the levels of valine adducts that about 3% of ethylene was metabolized to ethylene oxide. This is in close agreement with studies of ethylene metabolism in human volunteers, which determined an average conversion of 2% inhaled ethylene to ethylene oxide (Filser *et al.*, 1992). Analysis of inhaled ethylene and of adducts from ethylene oxide to N-terminal valine of hemoglobin were performed in 2 smokers to determine the percentage metabolism of ethylene to ethylene oxide due to smoking (Granath *et al.*, 1994). The results were comparable to previous metabolism studies; 2% inhaled ethylene was metabolized to ethylene oxide with a detoxification rate of $1\ hr^{-1}$ for ethylene oxide (corresponding to a $t_{1/2}$ of 42 min).

V. Effects of Animal Exposure

Taking into account the body weight and body surface differences between man and rats, the pharmacokinetics of ethylene in the two species were similar (Shen *et al.*, 1989). The concentration "body/air" ratio at steady state in rats was 0.54, indicating that accumulation in body tissues does not occur. Ethylene concentrations in rats were highest in fat and lowest in blood after a 12 hour exposure to 300 ppm ethylene (Eide *et al.*, 1995). Twelve hours after cessation of exposure, ethylene was not detectable in the fat. In another pharmacokinetic study in

rats, ethylene metabolism was found to follow first-order kinetics at atmospheric concentrations below 80 ppm (Filser and Bolt, 1984; Bolt and Filser, 1984). Above this range metabolism becomes increasingly saturated, reaching the maximum metabolic rate (V_{max}) at concentrations of 1000 ppm or more. In view of the saturability of ethylene metabolism, at which is found the maximal possible average body concentration of its metabolite, ethylene oxide, Bolt and Filser (1987) calculated that (theoretical) exposure of rats to ethylene at 40 ppm is equivalent to an ethylene oxide exposure of 1 ppm. However, because of the saturability of ethylene metabolism, ethylene concentrations of 1000 ppm or higher correspond to an ethylene oxide (theoretical) exposure of only 5.6 ppm. In a study of adduct formation among 1-alkenes, ethylene was found to produce a greater amount of hemoglobin and DNA adducts in rats (due to its metabolism to ethylene oxide) than other long-chain 1-alkenes (Eide *et al.*, 1995). In mice, S-(2-hydroxyethyl)cysteine was identified as a metabolite of ethylene in urine (3% of ^{14}C in urine) following inhalation of ^{14}C-ethylene (Ehrenberg *et al.*, 1977).

The available data indicate that ethylene has a low potential for non-cancer chronic toxicity in experimental animals.

In a 13-week inhalation study, 30 Sprague-Dawley rats/group/sex were exposed to 0, 300, 1000, 3000, or 10,000 ppm of ethylene for 6 hr/day, 5 days/week (Rhudy *et al.*, 1978; CIIT, 1977). Body weights, total weight gains and food consumption were not affected in any of the exposed animals. Hematology, clinical chemistry, urinalysis and histopathology did not find any treatment-related effects at any exposure level.

In a comprehensive lifetime inhalation study, 120 Fischer-344 rats/group/sex were exposed to ethylene concentrations of 0, 300, 1000 or 3000 ppm for 6 hr/day, 5 days/week, for up to 24 months (Hamm *et al.*, 1984; CIIT, 1980). Time-weighted average concentrations were 0, 301, 1003, and 3003 ppm. The maximum tolerated dose was not used as concentrations above 3000 ppm were hazardous due to ethylene's explosive properties. Over the 24 months, no differences were noted between exposure groups regarding mortality, clinical blood chemistry, urinalysis, body weights, organ weights or histopathology of a variety of tissues and organs. Inflammatory lesions typical of this strain of rat were distributed equally among all exposure groups.

VI. Derivation of Chronic Reference Exposure Level (REL)

Study	Schulte *et al.*, 1995 (for ethylene toxicity in humans, due to metabolism to ethylene oxide) Granath *et al.*, 1994; Filser *et al.*, 1992; Törnqvist *et al.*, 1989 (for determination of percentage ethylene conversion to ethylene oxide in humans)
Study population	28 or 10 Female U.S. hospital workers for the high and low exposure categories, respectively
Exposure method	Discontinuous whole body inhalation exposure (0.08 or 0.17 ppm ethylene oxide)
Critical effects	Reduced hematocrit and hemoglobin; elevated lymphocyte count; reduced neutrophil count; presence of hemoglobin adducts, micronuclei and SCEs in the blood.
LOAEL	0.17 ppm ethylene oxide
NOAEL	0.08 ppm ethylene oxide (3% conversion of ethylene to ethylene oxide in humans results in an estimated equivalent ethylene concentration of 2.67 ppm)
Exposure continuity	8 hours/day (10 m^3/day occupational inhalation rate), 5 days/week
Exposure duration	6.8 ± 5.5 years (mean ± 1 SD)
Average exposure	0.029 ppm for NOAEL group (ethylene oxide)
Human equivalent concentration	0.029 ppm for NOAEL group (ethylene oxide)
LOAEL uncertainty factor	1
Subchronic uncertainty factor	1
Interspecies factor	1
Intraspecies factor	10
Cumulative uncertainty factor	10
Inhalation reference exposure level for ethylene oxide	0.003 ppm (3 ppb)
Estimate of equivalent ethylene exposure	0.1 ppm ethylene (0.003 ppm /3%) (100 µg/m^3)

Two sets of evidence suggest that a NOAEL derived from ethylene oxide data may be the appropriate method to determine a human chronic inhalation REL for ethylene:

First, Bolt and Filser (1987) and Törnqvist (1994) have determined that the reason ethylene produces no non-cancer (and carcinogenic) effects in experimental animals is because ethylene's toxic metabolite, ethylene oxide, is never formed in high enough concentrations endogenously to cause a toxic effect. At high concentrations, ethylene is known to act acutely as a simple asphyxiant. But ethylene is also known to be metabolized to ethylene oxide, a known toxic agent

(both carcinogenic and non-cancer effects) in experimental animals. However, metabolism of ethylene to ethylene oxide becomes increasingly saturated as the concentration of ethylene approaches 1000 ppm. At saturation (above 1000 ppm), the theoretical ethylene oxide exposure is only 5.6 ppm. Exposure to 40 ppm ethylene was determined to be equivalent to an ethylene oxide exposure of 1 ppm. Long-term studies of ethylene oxide toxicity in experimental animals have determined the inhalation NOAEL to be 10-50 ppm (Lynch *et al.*, 1984a; Lynch *et al.*, 1984b). Thus, it may be impossible to obtain statistically significant non-cancer adverse effects with ethylene, regardless of dose. It is therefore not surprising that the long-term ethylene exposure study by Hamm *et al.* (1984) found no adverse effects.

Second, recent long-term studies of ethylene oxide inhalation by humans have indicated that humans may be more sensitive to ethylene oxide exposure than experimental animals. Non-cancer adverse effects (LOAELs) have been found at concentrations of 10 to 0.17 ppm (Zampollo *et al.*, 1984; Estrin *et al.*, 1987; Schulte *et al.*, 1995). In light of these findings, it is important to determine what percentage of ethylene is metabolized to ethylene oxide in humans. Several studies of ethylene metabolism in humans have determined that about 2-3% of ethylene, at low concentrations, is metabolized to ethylene oxide (Granath *et al.*, 1994; Törnqvist, 1994; Filser *et al.*, 1992; Törnqvist *et al.*, 1989). Since ethylene metabolism at low concentrations (1-50 ppm) follows first-order kinetics, the percentage of ethylene metabolism to ethylene oxide should remain the same at a range of chronic exposure levels. Therefore, the chronic REL for ethylene can be determined by analogy assuming 2-3% conversion of ethylene to ethylene oxide.

The NOAEL for ethylene oxide is based on the findings of Schulte *et al.* (1995), which found significant changes in hematological indices of female hospital workers at 0.17 ppm (LOAEL) but not at 0.08 ppm (time-weighted 8 hr exposure). Workers were exposed to ethylene oxide for an average of 5 years (range 0.5-10 years) Using 3% conversion of ethylene to ethylene oxide in humans and dividing this factor by the NOAEL established for ethylene oxide in humans, the NOAEL for ethylene exposure is 2.67 ppm. Adjusting the NOAEL to account for continuous 24-hour exposure yields a concentration of 0.89 ppm. An uncertainty factor of 10 is then applied to account for any increased susceptibility of sensitive human populations, resulting in an inhalation REL of 0.09 ppm for ethylene.

Pharmacokinetic studies in both man and experimental animals provide similar results: once inhaled ethylene is poorly absorbed into the bloodstream and is only minimally metabolized. Propylene, another small molecular weight olefinic gas, has similar pharmacokinetic properties in both man and animals. Ethylene oxide is the major metabolite of ethylene in both animals and man and is a carcinogen in laboratory animals.

The major strengths of the REL are the availability of long-term human exposure data on ethylene oxide (the toxic metabolite) and the observation of a NOAEL.

Weaknesses of the database for ethylene include the need to extrapolate potential health effects from metabolism data and a lack of multi-generation studies. Adverse effects on reproduction and development may occur where long-term chronic effects in adults have failed to reveal any

toxicity. Rats appear to be tolerant to the long-term effects of ethylene. Toxicity tests in a more ethylene-sensitive experimental animal would strengthen the database for ethylene.

VII. References

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CHRONIC TOXICITY SUMMARY

ETHYLENE DIBROMIDE

(1,2-Dibromoethane; dibromoethane; alpha, beta-dibromoethane; EDB; ethylene bromide; glycol bromide)

CAS Registry Number: 106-93-4

I. Chronic Toxicity Summary

Inhalation reference exposure level	**0.8 μg/m^3**
Critical effect(s)	Decreased sperm count/ejaculate, decreased percentage of viable and motile sperm, increased semen pH, and increased proportion of sperm with specific morphological abnormalities in human males
Hazard index target(s)	Reproductive system

II. Chemical Property Summary (HSDB, 1995)

Molecular formula:	$C_2H_4Br_2$
Molecular Weight:	187.88 g/mol
Description:	Colorless, heavy, nonflammable liquid with a mildly sweet, chloroform-like odor.
Vapor Pressure:	0.11 mm Hg at 20°C
Solubility:	Slightly soluble in water, 3400 mg/l water at 20°C. Miscible with most organic solvents.
Conversion factor:	7.68 μg/m^3 per ppb at 25°C

III. Major Uses and Sources

Ethylene dibromide (EDB) is used as a solvent for resins, gums and waxes, and as a chemical intermediate in the synthesis of dyes and pharmaceuticals (HSDB, 1995). EDB was once widely used as a fumigant for the control of pests in the U.S. Because of concerns regarding its carcinogenicity, the agricultural uses of EDB were banned in 1983 (RECT, 1988). EDB was also commonly used as a gasoline additive to scavenge inorganic lead compounds. The transition to the use of lead-free gasoline has drastically curtailed the use of EDB in this country (REPROTOX, 1995). EDB is now used mainly in industry. EDB may be formed naturally in the ocean as a result of macro algae growth. Exposure to the general population, via inhalation, may occur in the vicinity of industries and in industrial settings where this compound is manufactured

and used. Presently, exposure to EDB in ambient air from the use of leaded gasoline is expected to decrease dramatically.

IV. Effects of Human Exposures

Pharmacokinetic studies of EDB in humans could not be found in the literature. However, *in vitro* studies of EDB metabolism in human liver samples have been performed (Wiersma *et al.*, 1986). These experiments have shown that the enzyme systems known to metabolize EDB in rodent liver also metabolize EDB in the human liver. EDB was metabolized by human liver cytosolic glutathione S-transferases (GST), microsomal GST, and microsomal mixed function oxidases (MFO). MFO activity resulted in adducts irreversibly bound to protein while GST activity was mostly responsible for adducts irreversibly bound to DNA. Rodent liver enzymes similarly activate EDB to metabolites which bind to cellular macromolecules. In human fetal liver (16-18 weeks gestation) cytosolic GST was also found to metabolize EDB with high efficiency (Kulkarni *et al.*, 1992). Since detoxification via MFO activity may be limited at this stage of development, the results suggest that the human fetus may be at greater risk from EDB toxicity than adults.

A study of mortality from cancer and respiratory diseases was conducted among 161 employees exposed to EDB in 2 production units operated from 1942 to 1969 and from the mid-1920s to 1976, respectively (Ott *et al.*, 1980). No apparent connection was found between mortality due to respiratory diseases and exposure to EDB, when compared to U.S. white male mortality figures.

Due to the structural similarity of EDB to dibromochloropropane (DBCP), a known toxic agent in human male reproductive organs, a number of epidemiological studies concerning male reproduction and spermatogenesis were conducted:

In a study of 59 employees exposed to EDB at the Ethyl Corporation plant in Magnolia, Arkansas, the sperm counts of the exposed men were divided into 2 groups depending on estimated exposure (Ter Haar, 1980). Twenty percent of the low exposure group (<0.5 ppm) had sperm counts below 40 million, whereas 42% of the high exposure group (0.5 to 5 ppm) had sperm counts below this figure. The sperm counts were intermediate between counts reported for 2 types of U.S. samples (for normal men). The observed births among the two exposure groups were found to be similar to the number of expected births. The author determined that EDB had no effect on sterility or reproduction in the workers. Weaknesses of this study include the small population of exposed workers and the lack of a concurrent unexposed control group. Taking these defects of the study into account, a recent analysis concluded that the results provide evidence that EDB exposure between 0.5 and 5.0 ppm is associated with lower sperm counts (Dobbins, 1987).

A comparison of observed marital fertility with expected fertility (based on U.S. fertility rates) was conducted among 297 men working at 4 U.S. plants that manufacture EDB (Wong *et al.*, 1979). Fertility was 20% below expected for the four plants combined. This was largely due to

plant D which was 49% below the expected level. After omitting the incidence of vasectomies and hysterectomies among married couples, observed fertility was still 39% below the expected figure for plant D but was now no longer statistically significant. Exposure levels of EDB at plant D were not known but were estimated to be no more than 5 ppm. Later review determined that expected (control) levels of fertility and the power of the study were too low, resulting in the inability to identify a possible adverse effect (Dobbins, 1987). The lower fertility at plant D indicates that EDB has the potential to reduce fertility, but the extent of the reduction cannot be estimated from this study. Further treatment of the data by a method that uses the proper statistical adjustments of reproductive experience in the U.S. population (used as the control) suggests borderline significance for reduced fertility among the combined workers at the four plants (Wong *et al.,* 1985). The fertility evaluation indicates that more in-depth epidemiologic or physiologic studies are needed.

Semen analysis of 83 pineapple workers at two plantations was performed by Rogers and associates (1981). EDB-exposed workers were removed from each group and placed in a separate group. The remaining two groups of workers acted as control groups. Sperm count, motility and morphology were similar among the three groups. However, 43.8% of exposed workers had abnormally low counts (<40 million/ml) while abnormally low sperm counts of controls were 34.2% and 17.8%. Of the workers that had fertility tests done, 4/4 of the exposed workers tested in the infertile range. Forty percent or less tested in the infertile range among the control groups. The results suggest that workers exposed to EDB had reduced sperm counts, but exposure levels were not known.

Semen analysis among 46 men employed in the papaya fumigation industry was conducted to determine if EDB affected semen quality (Ratcliff *et al.*, 1987; Schrader *et al.*, 1987). Average duration of exposure was 5 years and the geometric mean breathing zone exposure to airborne EDB was 88 ppb (8 hr time weighted average) with peak exposures of up to 262 ppb. The comparison group consisted of 43 unexposed men from a nearby sugar refinery. Following consideration of confounding factors, statistically significant decreases in sperm count/ejaculate, the percentage of viable and motile sperm, and increases in the proportion of sperm with specific morphological abnormalities (tapered heads, absent heads, and abnormal tails) were observed among exposed men. Semen pH was significantly more alkaline than that of unexposed workers. Other measured sperm quality parameters were unchanged. This study suggests that EDB can result in reproductive impairment. However, no measurement of male fertility was conducted.

In a study that examined similar indices of semen quality, 6 week exposure of 10 forestry workers to EDB (60 ppb time weighted average, with peak exposures of up to 2165 ppb) resulted in decreased semen volume and slower sperm velocity (Schrader *et al.*, 1988). Six unexposed men were used as controls. The researchers suggest that short-term exposure to EDB results in decreased sperm velocity while long-term exposure, as in the previous study of EDB-exposed papaya workers, results in sperm immotility and cell death.

V. Effects of Animal Exposures

EDB is readily and rapidly absorbed from the lung when breathed as a vapor, the GI tract when taken orally, or through the skin when applied dermally (HSDB, 1995). In rats, the rate of absorption of EDB from the respiratory tract reached a plateau within 10 to 20 minutes following exposure to 75 ppm EDB for up to 2 hours (Stott and McKenna, 1984). About 58% of the EDB was absorbed. Intraperitoneal injection of [^{14}C]EDB into guinea pigs resulted in the highest concentrations in liver, kidneys and adrenals (Plotnick and Conner, 1976). Sixty-five percent of the dose was excreted as metabolites in urine, 3% in feces and 12% excreted unchanged in expired air. In rats, the highest concentrations of [^{14}C]EDB label were found in liver, kidney and spleen following an oral dose of 15 mg/kg body wt (Plotnick *et al.*, 1979). Studies with rats have provided evidence that 2 pathways of metabolic bioactivation exist for EDB (RECT, 1988). The oxidative pathway yields the metabolite 2-bromo-acetaldehyde, which is associated with cell macromolecule binding and liver damage. The conjugative pathway principally yields glutathione products, such as *S*-(2-bromoethyl)-glutathione, which are mainly responsible for DNA binding and mutagenesis. In rats, orally administered EDB is excreted primarily in the urine as mercapturic acid derivatives (Jones and Edwards, 1968). The biologic half-life for elimination of [^{14}C]EDB in rats is 5.1-5.6 hours (Watanabe *et al.*, 1978) and less than 48 hours in mice and guinea pigs (HSDB, 1995). Besides the small amount irreversibly bound to cell macromolecules and DNA, EDB shows little, if any, bioaccumulation in mammalian systems.

In a subchronic toxicity study of experimental animals, 19 rats and guinea pigs (up to 13 animals were rats and up to 4 were guinea pigs) were given EDB by oral administration for about 4 months (Aman *et al.*, 1946). Body weights and mortality of animals at or below an average daily dose of 40-50 mg/kg body wt-day were unaffected. However, only one control animal/species was used, the dosing regimen was not well described, and pathologic examination was apparently not performed.

Subchronic exposure of rats (20/sex/group) to 50 ppm EDB for as many as 63 seven-hour exposures in 91 days resulted in no significant change in body weights (Rowe *et al.*, 1952). Liver and kidney weights were increased in both sexes while testis weights were decreased in males. Also, lung weights in males were elevated and spleen weights in females were decreased. Histopathological examination revealed no changes. Guinea pigs (8/sex/group) subjected to as many as 57 seven-hour exposures of 50 ppm EDB in 80 days exhibited reduced body weights. Organ weights were unchanged, but microscopic examination of the livers showed slight central fatty degeneration. In kidneys, slight interstitial congestion and edema with slight parenchymatous degeneration of the tubular epithelium were observed. Four rabbits exposed to 59 seven-hour sessions at 50 ppm in 84 days showed no signs of adverse effects. Clinical signs of monkeys exposed to 50 ppm EDB (49 seven-hour exposures in 70 days) included an ill, unkempt appearance and nervousness. Slight central fatty degeneration in livers was observed, but pathology was not seen in other tissues. Exposure of the same four species to 25 ppm EDB for up to 220 days (145 to 156 seven-hour exposures) showed no signs of adverse effects.

In a 13-week inhalation study, 5 Fischer 344 albino rats/group/sex and 10 B6C3F1 mice/group/sex were exposed to 0, 3, 15 or 75 ppm EDB for 6 hr/day, 5 days/week (Reznik *et*

al., 1980). At 75 ppm, rats and mice exhibited severe necrosis and atrophy of the olfactory epithelium in the nasal cavity. Squamous metaplasia, hyperplasia and cytomegaly of the epithelium were also seen in nasal turbinals, larynx, trachea, bronchi and bronchioles. Minor alterations were seen in the nasal cavity of only a few male and female rats at 15 ppm. No compound-related lesions were observed in the olfactory and respiratory epithelium at 3 ppm. No lesions were seen in other tissues at any dose.

In another 13-week inhalation study, 40 male and 20 female CDF(F344) rats/group were exposed to 0, 3, 10 or 40 ppm EDB 6 hr/day, 5 days/week (Nitschke *et al.*, 1981). Male rats in the 40 ppm group exhibited decreased weight gain throughout most of the exposure period. However, reduced weight gain was never more than 6-8% below control levels. With the exception of decreased specific gravity of urine in females of the 40 ppm group, no treatment-related changes were observed in any rat group with respect to urinalysis, hematology and clinical chemistry. At the end of 13 weeks, relative liver and kidney weights of males exposed to 40 ppm EDB were significantly elevated while relative liver weights of females in the two highest exposure groups were significantly elevated. Absolute liver weight of females in the 40 ppm group were also significantly elevated. Histopathological examination revealed lesions primarily confined to the anterior sections of the nasal turbinates. Hyperplasia and nonkeratinizing squamous metaplasia of the respiratory epithelium were observed in nasal turbinates of rats exposed to 40 ppm EDB. Only slight epithelial hyperplasia of nasal turbinates was noted at 10 ppm. No treatment related effects were seen at 3 ppm. Livers of females in the 40 ppm group showed a slight increase in fat. After a 88 day recovery period, there was a reversion to normal of the nasal turbinates in all but one rat.

In what was originally scheduled to be a lifetime exposure study, 50 Osborne-Mendel rats/group/sex and 50 B6C3F1 mice/group/sex were administered EDB 5 days/week by gastric lavage over a substantial portion of their lifespan (NCI, 1978). Twenty untreated controls/sex and 20 vehicle controls/sex of each species were included in the study. Rats received initial doses of 80 and 40 mg/kg body wt-day for the first 17 weeks. Due to high mortality, dosing of high dose rats was discontinued for 13 weeks and resumed on week 30 at 40 mg/kg body wt-day. In week 42 all intubations of low and high dose rats ceased for 1 week followed by 4 weeks of dose administration. All surviving, treated male rats were sacrificed in week 49; all surviving, treated female rats were sacrificed in week 61. The resulting time-weighted average dosage over the test period was 38 and 41 mg/kg body wt-day for low and high dose males, respectively, and 37 and 39 mg/kg body wt-day for low and high dose females, respectively. Mice received initial dosages of 120 and 60 mg/kg body wt-day. In weeks 11-13, high and low dosages were increased to 200 and 100 mg/kg body wt-day, respectively. Original dosage levels were resumed after week 13. At week 40, administration of EDB was decreased to 60 mg/kg body wt-day for high dose mice. EDB administration was discontinued at week 54 with sacrifice occurring at week 78 for males and high dose females. Low dose female mice were observed for 37 weeks after intubation ceased. The resulting time-weighted average dosage over the test period was 62 and 107 mg/kg body wt-day for low and high dose mice, respectively. In rats, clinical signs by week 5 included reddened ears and hunched back in all treatment groups. By week 10, all treated rats had reduced body weights ($\geq$10%). Both female and male rats exhibited dose-dependent mortality. Many of the deaths occurred during or shortly after intubation, suggesting an acute

toxic reaction. Pathology revealed hyperkeratosis and acanthosis of the forestomach in high dose males and females and in one low dose female. A small number of rats in both treatment groups showed adrenal cortex degeneration and peliosis hepatitis of the liver. Dosed males showed early development of testicular atrophy. In mice, dose-related body weight reduction and mortality were observed. Clinical signs included alopecia, thin, hunched appearance, soft feces and body sores. Hyperkeratosis and acanthosis of the forestomach were seen in high dose male and female mice. One incidence each of hyperkeratosis (in a female) and acanthosis (in a male) was seen at the low dose. Splenic changes were present in high dose mice and testicular atrophy was present in high dose males.

In a long-term inhalation exposure study, F344 rats and $B6C3F_1$ mice were exposed to 0, 10 or 40 ppm EDB 6 hr/day, 5 days/week for up to 103 weeks (NTP, 1982). In male and female rats, the high dose groups had reduced body weights and increased mortality that began at about week 60. The treatment-related non-neoplastic pathology included hepatic necrosis (both sexes), epithelial hyperplasia and suppurative inflammation throughout the respiratory system (both sexes), and nephropathy (males only). Toxic nephropathy and mineralization were also seen in high dose female rats. Testicular degeneration and atrophy occurred with greater frequency in exposed rats but may be related to observed testicular tumors. Spermatic granulomas were also more frequently seen in high-dose males. Degeneration of the adrenal cortex appeared to be dose-related in females, but only one incidence each was seen in low and high dose males. Increased incidence of retinal atrophy was observed in exposed females. In mice, body weights were reduced at the high dose in both males and females. Many of the high dose animals exhibited a progressive weakness of the limbs or body during the second year. Increased mortality occurred in a dose-related manner in females and was significantly greater in low dose males. Non-neoplastic pathology included epithelial hyperplasia throughout the respiratory system and serous and suppurative inflammation of the nasal cavity in exposed mice. In all male mice, the principal cause of death was urinary bladder inflammation. However, bladder epithelial hyperplasia was only seen in exposed animals. An increased incidence of suppurative inflammation of the prostate was present but was also seen in controls. Dose-related spleen hematopoiesis was observed in females.

Another long-term inhalation study investigated the effects of 0 or 20 ppm EDB (7 hr/day, 5 days/week) on 48 Sprague-Dawley rats/sex/group for 18 months (Wong *et al.*, 1982). Significantly lower body weight gains (>10% difference from controls) occurred by the 15^{th} month in males, and by the 18^{th} month in females. Significantly reduced food consumption was not apparent. Increased mortality rates in both sexes occurred beginning in the 12^{th} month of EDB exposure. All hematological findings were within normal ranges. The only recorded non-neoplastic gross or microscopic finding was atrophy of the spleen in males, which may be related to tumor formation (hemangiosarcoma). The nasal cavity was not examined.

In a study of the effect of EDB on sperm production in bulls (Isreal-Friesian breed), 4 calves were fed 2 mg/kg body wt-day for 12 months (Amir and Volcani, 1965). The bulls were then given EDB in gelatin capsules every other day for 2-4 months longer. EDB did not appear to affect the growth, health and libido of the bulls. However, semen density and motility were significantly lower compared to untreated control bulls of the same age. Many abnormal

spermatozoa were also present in treated bulls. A NOAEL for this effect was apparently not determined. Cessation of EDB administration resulted in normal sperm within 10 days to 3 months. Further studies confirmed that EDB adversely affected sperm production without any other apparent effects on bulls (Amir and Volcani, 1967; Amir and Ben-David, 1973). However, feeding rams 2-5 mg/kg body wt-day for 120 days did not result in any effect on sperm or on the health of the animal (Amir, 1991).

In a developmental toxicity study, 15-17 pregnant Charles River CD rats and 17-19 pregnant CD mice were exposed to 0, 20, 38 and 80 ppm EDB by inhalation 23 hr/day during days 6 to 16 of gestation (Short *et al.*, 1978). A significant increase in mortality occurred in adult rats exposed to 80 ppm EDB and in adult mice exposed to 38 and 80 ppm EDB. Mice exposed to the highest dose experienced 100% mortality. Reduced body weights and feed consumption occurred in both species at all doses tested. Fetal mortality was increased in rats at the highest dose and in mice at 38 ppm. Reduced fetal body weights occurred at 38 ppm in rats and at all exposure levels in mice. No anomalies were seen in rat fetuses. An increase in runts at 38 ppm and a dose-dependent increase in skeletal anomalies were observed among mouse fetuses. However, these anomalies were characteristic of delayed development and occurred at doses that adversely affected maternal welfare. Therefore, these effects are indicative of fetal toxicity rather than teratogenicity.

Male reproductive toxicity of EDB has been evaluated in some other experimental animals. New Zealand white rabbits dosed subcutaneously with 0, 15, 30 or 45 mg/kg body wt-day resulted in adverse effects at the highest dose (Williams *et al.*, 1991). Increased mortality, increased serum enzymes, and liver damage were observed at this dose level. With respect to sperm quality, sperm velocity, motility, and motion parameters were reduced at the highest dose. A dose related decrease in semen pH was also noted. However, male fertility and fetal structural development were unaffected.

In contrast, the dominant lethal assay in mice was negative following a single intraperitoneal injection of 100 mg EDB/kg body wt (Barnett *et al.*, 1992). Germ cell tests did not indicate that EDB was a germ cell mutagen in male mice.

VI. Derivation of Chronic Reference Exposure Level (REL)

Study	Ratcliff *et al.*, 1987
Study population	46 exposed men, 43 unexposed men; 89 total
Exposure method	Variable workplace breathing zone airborne exposure (88 ppb geometric mean 8-hour time weighted average exposure with peak exposures up to 262 ppb)
Critical effects	Reproductive toxicity; decreased sperm count/ejaculate, decreased percentage of viable and motile sperm, increased semen pH, and increased proportion of sperm with specific morphological abnormalities (tapered heads, absent heads, and abnormal tails) in human males
LOAEL	0.088 ppm
NOAEL	Not observed
Exposure continuity	8 hr/day (10 m^3/day occupational exposure rate), 5 days/week
Exposure duration	Average, 4.9 years (with standard deviation of 3.6 years)
Average experimental exposure	0.031 ppm for LOAEL group
Human equivalent concentration	0.031 ppm
LOAEL uncertainty factor	10
Subchronic uncertainty factor	3
Interspecies factor	1
Intraspecies factor	10
Cumulative uncertainty factor	300
Inhalation reference exposure level	0.0001 ppm (0.1 ppb, 0.0008 mg/m^3, 0.8 μg/m^3)

The primary study by Ratcliff and associates (1987) found significant changes in sperm quality indices of papaya workers exposed to EDB vapors for an average of nearly 5 years. No other health effects were apparent. A level of EDB at which no toxicity was observed (NOAEL) was not determined.

In addition to the primary study of Ratcliff *et al.* (1987), several other epidemiological studies strongly suggest a correlation between EDB exposure and male reproductive toxicity (Ter Haar, 1980; Wong *et al.*, 1979; Wong *et al.*, 1985; Rogers *et al.*, 1981; Schrader *et al.*, 1988). This lesion appears to occur in humans at concentrations at which other toxic effects are not seen. EDB also shares some structural similarity to dibromochloropropane (DBCP), a known reproductive toxicant in human males. The evidence for male reproductive toxicity of EDB is not as strong as that for DBCP, probably because EDB is not as potent as DBCP for producing this toxic effect. However, the number of studies indicating a connection between male reproductive toxicity and EDB exposure cannot be ignored for the development of the REL.

Chronic oral exposure of bulls to EDB results in similar toxic effects at low concentrations (equivalent to 0.9 ppm) without affecting the general health of the animal (Amir and Volcani, 1965; Amir, 1991). Unfortunately, a dose-response effect for EDB toxicity, as well as a determination of the NOAEL, has yet to be determined in bulls. Long-term studies of EDB toxicity in other experimental animals suffer from some of the same data deficiencies. Two lifetime studies of EDB exposure in rodents did not observe a NOAEL (NCI, 1978; NTP, 1982). Evidence of testicular atrophy was found in both studies, but at concentrations that also produced toxic effects in other organ systems. The database for chronic toxicity of EDB in experimental animals would be enhanced if the proper doses were chosen to determine a NOAEL.

The strengths of the inhalation REL include the use of human exposure data from workers exposed over a period of years. Major areas of uncertainty are the lack of observation of a NOAEL, the uncertainty in estimating exposure, the potential variability in exposure concentration, and the limited nature of the study (fertility was not actually tested).

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CHRONIC TOXICITY SUMMARY

ETHYLENE DICHLORIDE

(1,2-Dichloroethane)

CAS Registry Number: 107-06-2

I. Chronic Toxicity Summary

Inhalation reference exposure level	**400 µg/m^3**
Critical effect(s)	Hepatotoxicity; elevated liver enzyme levels in serum of rats.
Hazard index target(s)	Alimentary system; nervous system

II. Physical and Chemical Properties (HSDB, 1995)

Molecular formula	$C_2H_4Cl_2$
Molecular weight	98.97 g/mol
Description	Clear, colorless oily liquid
Specific gravity	1.2351 @ 20ºC
Boiling point	83.5ºC
Vapor pressure	64 torr @ 20ºC
Solubility	Slightly soluble in water (0.869 g/100 ml at 20ºC); miscible with alcohol; soluble in ordinary organic solvents
Conversion factor	1 ppm = 4.05 mg/m^3

III. Major Uses or Sources

Ethylene dichloride (EDC) is used primarily in the production of vinyl chloride monomer (HSDB, 1995). It is also an intermediate in the manufacture of trichloroethane and fluorocarbons. EDC has been used as a solvent and a soil fumigant.

IV. Effects of Human Exposure

Nausea, vomiting, dizziness, and unspecified blood changes were reported in a study of workers exposed to levels of 10-37 ppm EDC (Brzozowski *et al.*, 1954). Kozik (1957) reported adverse central nervous systems and liver effects in workers occupationally exposed to concentrations of 16 ppm EDC and below. Nervous system effects were also reported by Rosenbaum (1947) in a

study of 100 Russian workers exposed for less than 5 years to concentrations of EDC less than 25 ppm.

Immediately following a 30-minute exposure to an unknown concentration of EDC, a 51 year-old male was somnolent and experienced vomiting (Nouchi *et al.*, 1984). Delirious and trembling, the worker was admitted to the hospital 20 hours post-exposure; the liver was palpable, but serum liver enzymes were normal. The patient lapsed into a coma 3.5 hours following admission to the hospital. A marked elevation in serum liver enzymes was noted on the second day of hospitalization, 35 hours post-exposure. Multiple organ failure occurred on the fourth day of hospitalization and the patient died of arrhythmia. At autopsy, the lungs were congested and edematous. Diffuse degenerative changes were observed in the myocardium. Extensive centrilobular necrosis was observed in the liver and acute centrilobular necrosis was observed in the kidney. Nerve cells in the brain, including Purkinje cells, appeared shrunken with pyknotic nuclei. The latency period for hepatotoxicity of approximately 20 hours suggests that metabolism of the compound yields the reactive agent.

V. Effects of Animal Exposure

Male and female rats (50 per sex) were exposed to 50 ppm EDC 7 hours per day, 5 days per week for 2 years (Cheever *et al.*, 1990). The rats were 5.5-6 weeks of age at the beginning of exposure. No significant increases in any tumor type were observed. Absolute and relative liver weights were not significantly different from controls.

Rats (8-10 per sex per group) were exposed to 0, 5, 10, 50, and 150-250 ppm EDC 7 hours per day, 5 days per week for up to 18 months (Spreafico *et al.*, 1980). Serum chemistry measurements were taken after 3, 6, 12, and 18 months of exposure. Rats to be examined after 3, 6 and 18 months of exposure were 3 months of age at the beginning of the experiment, and rats to be examined after 12 months of exposure were 14 months of age at the beginning of the experiment. No significant changes in serum chemistry parameters were observed at 3, 6, or 18 months of exposure. Rats exposed to EDC for 12 months exhibited changes in serum chemistry indicative of chronic liver damage. Most notably, significant increases in alanine aminotransferase (ALT), lactate dehydrogenase (LDH) and uric acid were observed in addition to significant decreases in cholesterol and aspartate aminotransferase (AST). Blood urea nitrogen (BUN) and 3-glutamyl transpeptidase were also elevated but at non-significant levels. At 150 ppm, similar changes were observed with a statistically significant elevation in BUN. At lower concentrations, AST was significantly elevated while ALT was within normal range. The marked difference between serum chemistry parameters following 12 months of exposure compared to those following 3, 6 and 18 months of exposure may be due to the considerable difference in age of the rats at the start of exposure. This study identifies a 12-month LOAEL of 50 ppm and a NOAEL of 10 ppm in rats.

A study examining the interaction between 1,2-dichloroethane and disulfiram (DSF), a non-carcinogen used extensively in the rubber industry and as a treatment for alcoholism, exposed rats to EDC concentrations of 300 ppm and greater 5 days per week for 30 days (Igwe *et*

al., 1986). Increased liver weights were observed in rats following exposure to 450 ppm EDC (the LOAEL for this study). This study also determined that the interaction between DSF and EDC greatly increased the toxicity of EDC. Therefore, any person exposed to DSF either occupationally or therapeutically is likely to be more susceptible to the effects of EDC toxicity.

Rats, rabbits, guinea pigs, dogs, cats and monkeys were used in exposures ranging from approximately 100 to 1000 ppm EDC (Heppel *et al.*, 1946). At the highest experimental concentration of 963 ppm, high mortality was observed in rats, rabbits, and guinea pigs following exposure 7 hours per day, 5 days per week for two weeks or less. Guinea pigs exposed to this concentration exhibited lacrimation and inactivity during exposure; pulmonary congestion was noted at autopsy. Rats exposed to this concentration exhibited degenerative proliferative changes in the renal tubular epithelium and splenitis. Pulmonary congestion and focal hemorrhage were also noted in 2 of 4 rats examined. While 4 of 6 cats exposed to this concentration survived until sacrifice 11 weeks following termination of exposure, congestion and fatty infiltration of the liver were observed at necropsy. Due to high mortality in the rodents at the higher concentration, a subsequent experiment exposed rats and guinea pigs 7 hours per day, 5 days per week to 100 ppm EDC for four months. No increase in mortality or effects on growth were observed in rats exposed to this concentration. The rats were successfully bred and their pups were exposed with the dams. No significant findings were observed upon gross and histological examinations of 10/39 exposed and 10 control rats. This study is severely limited by the methods of determining the exposure concentration and by the lack of quantitative measurements of toxicity other than death. This study does however indicate that fatty infiltration of the liver is one indication of toxicity following multiple exposures to EDC.

A comparative study of the toxicity of EDC administered 0, 500, 1000, 2000, 4000, and 8000 ppm in drinking water to several species of rats for 13 weeks (Morgan *et al.*, 1990). A statistically significant increase in kidney weight was observed in male and female F344/N rats administered 1000 ppm or greater in drinking water. A statistically significant decrease in body weight was observed in rats administered 8000 ppm. Significant decreases in absolute and relative kidney weight were observed in male and female rats administered concentrations of 1000 ppm EDC. A significant increase in relative liver weight was observed in male rats administered 2000 ppm EDC and greater and female rats administered 4000 ppm EDC and greater. Similar but less marked toxicity was observed in the Sprague-Dawley and Osborne-Mendel rats administered 1000 ppm. Additionally, rats were administered EDC in corn oil by gavage at doses of 0, 30, 60, 120, 240, and 480 mg/kg for 13 weeks. Rats administered EDC by gavage exhibited high mortality in the higher dose groups. Statistically significant increases in kidney weights were observed in surviving male rats administered EDC and in female rats administered 120 or 240 mg/kg.

VI. Derivation of Chronic Reference Exposure Level (REL)

Study	Spreafico *et al.*, 1980.
Study population	Rats (8-10 per sex/group)
Exposure method	Discontinuous whole-body inhalation exposures (0, 5, 10, 50, or 150-250 ppm)
Critical effects	Significant elevation in liver enzymes
Exposure duration	12 months
Exposure continuity	7 hours/day, 5 days/week
LOAEL	50 ppm
NOAEL	10 ppm
Average experimental exposure	2.1 ppm for NOAEL group (50 x 7/24 x 5/7)
Human equivalent concentration	3.2 ppm for NOAEL group (gas with systemic effects, based on RGDR = 1.5 for lambda (a) : lambda (h)) (Gargas *et al*., 1989)
LOAEL uncertainty factor	1
Subchronic uncertainty factor	1
Interspecies uncertainty factor	3
Intraspecies uncertainty factor	10
Cumulative uncertainty factor	30
Inhalation reference exposure level	0.1 ppm (100 ppb; 0.4 mg/m^3; 400 $\mu g/m^3$)

The strengths of the inhalation REL include the availability of chronic inhalation exposure data, the relatively large number of exposure levels at lower concentrations (allowing for better elucidation of the dose-response relationship for hepatotoxicity), and the observation of a NOAEL.

Major areas of uncertainty are the lack of adequate human exposure data, the lack of reproductive and developmental toxicity studies, the small groups tested in the study, and the lack of multiple-species health effects data.

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CHRONIC TOXICOLOGY SUMMARY

ETHYLENE GLYCOL

(1,2-dihydroxyethane; 1,2-ethanediol)

CAS Registry Number: 107-21-1

I. Chronic Toxicity Summary

Chronic reference exposure level	**400 μg/m^3**
Critical effects	Eye and respiratory irritation in human volunteers
Hazard index target(s)	Respiratory system; eyes; kidney; teratogenicity

II. Physical and Chemical Properties (HSDB, 1996)

Description	Clear, colorless, odorless liquid
Molecular formula	$C_2H_6O_2$
Molecular weight	62.07 g/mol
Specific gravity	1.1135 @ 20° C
Boiling point	197.6° C
Vapor pressure	0.06 mm Hg @ 20° C
Solubility	Soluble in water and ethanol; slightly soluble in ether. Insoluble in benzene and petroleum ether.
Conversion factor	1 ppm = 2.5 mg/m^3 @ 25° C

III. Major Uses and Sources

Ethylene glycol is used as an antifreeze agent in cooling and heating systems (HSDB, 1996). It is used in hydraulic brake systems; as an ingredient in electrolytic condensers; as a solvent in the paint and plastics industries; in inks for ball-point pens and printer's inks. It is used in the manufacture of some synthetic fibers (Terylene and Dacron), and in synthetic waxes. It is a vehicle for some pharmaceutical preparations. It is used in some skin lotions and flavoring essences. Also, it is used in asphalt emulsion plants, in wood stains and adhesives, and in leather dyeing. It has been used as a de-icing fluid for airport runways.

IV. Effects of Human Exposure

Laitinen *et al.* (1995) found that 10 motor servicing workers had significantly higher urinary levels of ethylene glycol and ammonia, and decreased urinary glycosaminoglycan levels, compared with 10 controls. The ethylene glycol levels in air were undetectable in the workers' breathing zones (i.e. below 1.9 ppm), therefore dermal absorption appeared to be the primary route of exposure. Because the dermal absorption rate is high, airborne ethylene glycol concentrations in workplaces likely underestimate the total exposure.

In a study of 20 volunteer male prisoners, 20 hour/day exposure to ethylene glycol concentrations of up to 20 ppm (49 mg/m^3) for 30 days was without effect (Wills *et al.*, 1974). Irritation was noted after 15 minutes at an exposure concentration of 75 ppm (188 mg/m^3), and became quickly intolerable at 123 ppm (308 mg/m^3). No effects were observed in clinical serum enzyme levels for liver and kidney toxicity, hematotoxicity, or psychological responses. The irritation resolved soon after exposure with no long term effects noted after a 6-week follow-up period.

V. Effects of Animal Exposure

A chronic feeding study in rats and mice was conducted by DePass *et al.* (1986a). In this study, rats (130 per sex per group) and mice (80 per sex per group) were exposed to 0, 0.04, 0.2, or 1 g/kg/day for up to 2 years. All male rats in the high dose group died by 475 days. A large number of effects were observed in this group, including: reduced body weight, increased water intake, increased blood urea nitrogen and creatinine, reduced erythrocyte counts, reduced hematocrit and hemoglobin, increased neutrophil count, and increased urine volume. Heart, kidney, lung, parathyroid, stomach, and other vascular mineralization and hyperplasia were observed histologically in the high dose group of the male rats. Female rats exhibited fatty changes and granulomas in the liver at the high dose. Liver effects were not reported for the males. The NOAEL in rats for chronic oral ethylene glycol toxicity was 200 mg/kg/day. No effects were observed in mice. Therefore, the NOAEL for mice was 40 mg/kg/day.

Studies on the effects of inhaled ethylene glycol on reproduction and development of rats and mice were conducted by Tyl *et al.* (1995a, 1995b). In a study using whole-body exposure of rats and mice to ethylene glycol at analyzed concentrations of 0, 119, 888, or 2090 mg/m^3 for 6 hours/day on days 6-15 of gestation, mice were found to be the more sensitive species. Maternal toxicity in rats included a significant increase in absolute and relative liver weight at 2090 mg/m^3. No effects on weight gain, organ weights other than liver, fecundity, live fetuses per litter, or pre- or post-implantation loss were observed in rats. In addition, terata were not observed at any concentration. Reduced ossification in the humerus, zygomatic arch, and the metatarsals and proximal phalanges of the hindlimb was present in fetuses exposed to 888 or 2090 mg/m^3. The NOAEL for maternal toxicity in rats was 888 mg/m^3, while the NOAEL for fetotoxicity was 119 mg/m^3.

In mice, reduced body weight and gravid uterine weight during and after the exposure were observed at the 888 and 2090 mg/m^3 concentrations. Increased nonviable implants per litter and

reduced fetal body weights were also observed in groups exposed to 888 or 2090 mg/m^3. External, visceral, skeletal, and total malformations were increased in the 888 and 2090 mg/m^3 groups. The NOAEL for these effects in mice was 119 mg/m^3.

A similar experiment in mice using nose-only exposures was conducted by these researchers (Tyl *et al.*, 1995a) to determine the role of dermal absorption and/or ingestion on the effects observed with the whole-body exposure. Nose-only exposures to ethylene glycol were for 6 hours/day, on gestational days 6 through 15 at concentrations of 0, 500, 1000, and 2000 mg/m^3. The NOAEL for maternal effects (increased kidney weight) was 500 mg/m^3, and the NOAEL for fetal toxicity (skeletal variations and fused ribs) was 1000 mg/m^3. Thus, secondary dermal and/or oral exposures appear to have contributed significantly to the developmental and maternal toxicity in mice exposed to ethylene glycol aerosol. The nose-only inhalation exposure study by Tyl *et al.* (1995) was conducted in addition to the whole-body inhalation study since extensive adsorption of ethylene glycol onto the fur of the animals was demonstrated in the whole-body experiment. Normal grooming behavior would have resulted in significantly larger doses of ethylene glycol than that expected by inhalation only.

A 3-generation study on the effects of ethylene glycol on reproductive performance and gross health of offspring in rats was conducted by DePass *et al.* (1986b). Rats were exposed orally to 40, 200, or 1000 mg/kg/day ad libitum in the feed through 3 generations. No effects on pup survivability or pup body weight were observed. Total and viable implants were also not affected. Teratogenic effects were not examined in this study.

Tyl *et al.* (1993) studied the reproductive and developmental effects of ethylene glycol in rabbits exposed by gavage on days 6 to 19 of gestation. Dams were exposed to 0, 100, 500, 1000, or 2000 mg/kg/day. Exposure to 2000 mg/kg/day resulted in 42% mortality, and abortion or early delivery in 4 does. No evidence of embryotoxicity or teratogenicity was observed in the groups exposed to 1000 mg/kg/day or less. The NOAEL for maternal toxicity was determined to be 1000 mg/kg/day.

VI. Derivation of Chronic Reference Exposure Level

Study	Wills *et al.* (1974)
Study population	Human volunteer prisoners
Exposure method	Discontinuous whole-body inhalation
Critical effects	Eye and respiratory tract irritation
LOAEL	75 ppm
NOAEL	20 ppm
Exposure continuity	20 hours/day
Exposure duration	30 days
Average exposure	16.7 ppm for NOAEL group (20 x 20/24)
Human equivalent concentration	16.7 ppm
LOAEL uncertainty factor	1
Subchronic uncertainty factor	10
Interspecies factor	1

Intraspecies factor	10
Cumulative uncertainty factor	100
Inhalation reference exposure level	0.2 ppm (200 ppb; 0.4 mg/m^3; 400 μg/m^3)

The subchronic study by Wills *et al.* (1974) represents the only human inhalation data for ethylene glycol toxicity. The experiment showed a concentration-response relationship, with onset of irritation occurring at 188 mg/m^3 and intense and intolerable irritation occurring at 308 mg/m^3. The volunteers were followed for 6 months without any apparent long-term effects from the exposures. Although the irritation experienced in the human subjects appears to be an acute phenomenon and not a cumulative lasting effect, the subchronic uncertainty factor was retained to protect against other systemic effects which may occur over a long-term exposure.

The chronic feeding study in rats by DePass *et al.* (1986a) showed significant chronic effects including reduced body weight, increased water intake, increased blood urea nitrogen and creatinine, reduced erythrocyte counts, reduced hematocrit and hemoglobin, increased neutrophil counts, increased urine volume, and reduced urine specific gravity and pH in rats exposed to a concentration of 1000 mg/kg/day. However, no effects were reported in mice. In contrast, reproductive and developmental toxicity studies in mice, rats, and rabbits have shown the mouse to be the more sensitive species for both terata and maternal toxicity endpoints (Tyl *et al.*, 1995a; Tyl *et al.*, 1993, Neeper-Bradley *et al.*, 1995). In addition, the 3-generation reproductive toxicity study by DePass *et al.* (1986b) showed no significant effects on rat pup survival or body weight at concentrations up to 1000 mg/kg/day. However, developmental endpoints were not reported in this study. From the available data, the toxicity of ethylene glycol is apparently greatest in the maternal mouse. The estimated equivalent air concentrations (assuming a 70 kg human inhales 20 m^3/day) from the feed in the 3-generation study by DePass *et al.* (1986b) are 700 mg/m^3 and 3500 mg/m^3 for the NOAEL and LOAEL, respectively. If RELs were estimated from this study or other animal studies, they would essentially be the same or higher than those calculated based on the human study.

The strengths of the inhalation REL include the use of human exposure data, the use of controlled inhalation exposures, and the observation of a NOAEL. A major area of uncertainty is the lack of chronic inhalation exposure studies.

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CHRONIC TOXICITY SUMMARY

ETHYLENE GLYCOL MONO-N-BUTYL ETHER

(EGBE; butoxyethanol; BE; Butyl Cellosolve®; butyl glycol; butyl glycol ether)

CAS Registry Number: 111-76-2

I. Chronic Toxicity Summary

Inhalation reference exposure level	**200 µg/m^3**
Critical effect(s)	Hematological effects in rats
Hazard index target(s)	Circulatory system

II. Chemical Property Summary (HSDB, 1995)

Molecular formula	$C_6H_{14}O_2$
Molecular weight	118.2 g/mol
Description	Colorless liquid
Vapor pressure	0.76 mm Hg @ 20°C
Solubility	Sol. in 20 parts water; miscible in most organic solvents
Conversion factor	4.83 µg/m^3 per ppb at 25ºC

III. Major Uses and Sources

Ethylene glycol mono-n-butyl ether (EGBE) is highly miscible with water and oil and therefore has numerous industrial and household uses as a solvent or cleaner. As of 1983, EGBE was the largest volume glycol ether produced (~10^8 kg/yr, HSDB, 1995). EGBE has uses as a solvent for protective coatings and metal cleaners, a component of hydraulic fluids, a chemical intermediate in the synthesis of di(2-butoxyethyl)phthalate plasticizer and 2-butoxyethyl acetate, and as a coupling agent to stabilize immiscible components of water-based coatings, textile lubricants, and cutting oils.

An approximate breakdown of EGBE use is 41% as a solvent in protective coatings, 18% as a solvent in metal and liquid household cleaners, 10% in the synthesis of 2-butoxyethyl acetate and di(2-butoxyethyl)phthalate, and 31% in other solvent uses (HSDB, 1995).

IV. Effects of Human Exposure

The only studies available which address the toxicity of ethylene glycol monobutyl ether to humans are case reports of toxicity from occupational exposure by inhalation or ingestion and a single study of effects related to short term exposure by inhalation (Carpenter *et al.*, 1956). In that study, six volunteer subjects were exposed to concentrations ranging from 98 to 195 ppm EGBE for 4 or 8 hours. Observations noted at all levels included irritation of the eyes and nose, runny nose, taste disturbances and, in one subject, vomiting. All three subjects exposed to 195 ppm EGBE agreed that this level caused discomfort. Based upon the studies of Werner *et al.* (1943), in dogs showing increased erythrocyte fragility in vitro, Carpenter also examined erythrocyte fragility in vivo, but did not observe this effect in humans at the EGBE exposure levels studied.

Although increased erythrocyte fragility has been observed in rodents following exposure to EGBE (Carpenter *et al.*, 1956) recent studies found no increase in the fragility of human erythrocytes taken from normal and susceptible individuals (persons with hereditary spherocytosis or sickle cell disease and older persons) following a 4-hour incubation with butoxyacetic acid, the presumed EGBE metabolite responsible for hematotoxicity (Udden, 1994; Udden and Patton, 1994; Ghanayem *et al.*, 1987; Ghanayem, 1989).

V. Effects of Animal Exposure

Experiments were conducted evaluating the toxicity of inhaled EGBE in Fischer 344 rats, including 9-day, and 90-day exposure regimens (Dodd *et al.*, 1983). In the subchronic (90-day) portion of the study, 10 rats/sex/dose group were exposed for 6 hrs/day, 5 days/wk for 13 weeks (66 exposures) to analytical concentrations of 0, 4.7, 25, or 77 ppm EGBE. Another subset of 6 rats/sex/dose group were exposed simultaneously for 6 weeks. No significant effects on body weight, organ weights, clinical chemistry or urine composition were identified, nor were any gross or microscopic lesions. The only significant changes observed were a slight decrease in red blood cells (RBC) among male and female rats and a slight increase in mean corpuscular hemoglobin (MCH) among female rats in the high dose group. Among female rats, the decrease in RBC was more pronounced after 31 complete exposures. Animals (6/sex/dose) in the 9-day study were exposed for 6 hr/day for 5 consecutive days and then for 4 more consecutive days (following a 2 day break) to nominal concentrations of 0, 20, 86, and 245 ppm EGBE. Among animals in the highest dose group audible respiration and nasal discharge were observed during exposure. Weight gain was also depressed in these animals, but returned to normal values during a two week recovery period. Animals in the highest dose group showed hematological toxicity including decreased RBCs, hemoglobin, and mean corpuscular hemoglobin. Decreased hemoglobin was also observed in the 86 ppm dose group, but the effect was not as pronounced as in the highest dose group. No significant changes were observed in the animals exposed to 20 ppm for 9 days.

Dogs (2/group) were exposed to 415 ppm EGBE for 7 hours/day, 5 days/week for 12 weeks (Werner *et al.*, 1943a). Weight losses of 6 and 9% were reported in exposed animals.

Hematological effects included decreased hemoglobin, erythrocytes, and hematocrit. Effects observed, but not quantitated, included increased microcytosis, hypochromia, and poly-chromatophilia. Changes in hematological parameters in the control animals during the course of the experiment made determination of compound-related effects difficult. The same group reported on the toxicity of EGBE to rats (23/group) exposed to 135 or 320 ppm EGBE for 5 hours/day, 5 days/week for 1, 3, or 5 weeks, including one group sacrificed 1 week post-exposure (Werner *et al.*, 1943b). In both dose groups, erythrocyte count and hemoglobin concentrations were decreased and reticulocyte count was increased.

Rats (4/sex/dose group) were exposed to 0, 20, 50, or 100 ppm EGBE to 15 exposures of 6 hours/day (Gage, 1970). Rats in the 100 ppm EGBE dose group showed increased erythrocyte fragility. No effects were observed in animals in lower dose groups.

Pregnant rats and rabbits (36 and 24 dams/dose group, respectively) were exposed by inhalation to EGBE at concentrations of 0, 25, 50, 100, or 200 ppm for gestational days 6-15 in the rats and 6-18 in the rabbits (Tyl *et al.*, 1984). Maternal toxicity was observed in rats at 100 and 200 ppm EGBE with decreased weight gain, changes in organ weight, changes in food consumption, indications of anemia, and clinical signs including eye wetness and nasal encrustation among the observed adverse effects. Rabbit dams showed signs of toxicity at 200 ppm EGBE, with two deaths reported during the exposure or post-exposure period and decreased weight and some clinical signs including eye and nose wetness and stained fur.

In a study examining the teratological effects from inhalation of 0, 150, or 200 ppm EGBE on pregnant rats, maternal toxicity was observed (Nelson *et al.*, 1984). Animals (N = 18 or 19 with 34 control animals) were exposed for 7 hours/day on gestational days 7-15 and sacrificed on day 20. Hematuria was noted on the first day, but not on subsequent days, in animals exposed to 150 and 200 ppm EGBE. No significant dose-related effects were observed with respect to developmental endpoints.

VI. Derivation of Chronic Reference Exposure Level (REL)

Study	Dodd *et al.*, 1983
Study population	Rats
Exposure method	Discontinuous inhalation exposure
Critical effects	Decreased red blood cells in females
LOAEL	77 ppm
NOAEL	25 ppm
Exposure continuity	6 hr/day, 5 days/week
Exposure duration	13 weeks
Average experimental exposure	4.5 ppm for NOAEL group (25 x 6/24 x 5/7)
Human equivalent concentration	4.5 ppm for NOAEL group (gas with systemic effects, based on RGDR = 1.0 using default assumption that lambda (a) = lambda (h))
LOAEL uncertainty factor	1

Subchronic uncertainty factor	10
Interspecies factor	1
Intraspecies factor	10
Cumulative uncertainty factor	100
Inhalation reference exposure level	0.04 ppm (40 ppb, 0.2 mg/m^3, 200 µg/m^3)

The hematopoietic system toxicity from inhalation of EGBE has been clearly established from laboratory studies of animals (Dodd *et al.*, 1983; Werner *et al.*, 1943a; Werner *et al.*, 1943b; Gage, 1970; Carpenter *et al.*, 1956). Two studies report NOAELs of 23 ppm EGBE (Dodd *et al.*, 1983) and 50 ppm EGBE (Gage, 1970). The studies of Werner (1943a, 1943b) produced LOAELs of 415 ppm and 135 ppm EGBE, without an observed NOAEL. The lowest NOAEL comes from the study of Dodd *et al.* (1983) showing effects of EGBE on red blood cell levels in a 13-week study. Although only a single dose in the study produced the effect in both sexes (72 ppm EGBE), the shorter term (9 days) study also showed this effect. This value is thus accepted as the basis for the derivation of the chronic REL.

NOAELs of 50 and 100 ppm EGBE were observed in pregnant rats and rabbits exposed by inhalation to EGBE, respectively, with the appearance of maternal toxicity (decreased weight gain, changes in organ weight, changes in food consumption, hematuria, indications of anemia, etc.) in the 100 and 200 ppm EGBE dose groups (Tyl *et al.*, 1984). The proximity of these levels to those producing hematological effects suggests that both endpoints should be of concern near the chronic REL.

No uncertainty factor was applied for interspecies extrapolation in light of some evidence that humans are not more sensitive than experimental animals for hematological effects (Udden, 1994; Udden and Patton, 1994; Ghanayem *et al.*, 1987; Ghanayem, 1989).

The strengths of the inhalation REL include the availability of controlled inhalation exposure data at multiple exposure concentrations and the observation of a NOAEL. Major areas of uncertainty are the lack of adequate human exposure data and the lack of chronic, multiple-species health effects data.

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CHRONIC TOXICITY SUMMARY

ETHYLENE GLYCOL MONOETHYL ETHER

(2-ethoxyethanol; EGEE)

CAS Registry Number: 110-80-5

I. Chronic Toxicity Summary

Inhalation reference exposure level	**200 µg/m^3** (US EPA RfC) This document summarizes the evaluation of non-cancer health effects by US EPA for the RfC
Critical effect(s)	Testicular degeneration and decreased hemoglobin in rabbits
Hazard index target(s)	Reproductive system; circulatory system

II. Chemical Property Summary (from HSDB, 1995, except as noted)

Molecular formula	$C_4H_{10}O_2$
Molecular weight	90.12
Description	Colorless liquid
Vapor pressure	3.8 mm Hg @ 20° C (ACGIH, 1991)
Solubility	Miscible with water and organic solvents
Conversion factor	3.69 µg/m^3 per ppb at 25ºC

III. Major Uses and Sources

Ethylene glycol monoethyl ether (EGEE) is a widely used solvent for nitrocellulose, dyes, inks, resins, lacquers, paints, and varnishes (HSDB, 1995). It is also a component of many cleaning agents, epoxy coatings, paints, hydraulic fluid, and is an anti-icing fuel additive in aviation. EGEE is also a chemical intermediate in the production of another solvent, ethylene glycol monoethyl ether acetate.

IV. Effects of Human Exposure

Sperm quality was examined in 37 workers exposed to EGEE by skin contact and/or inhalation in two buildings (Clapp *et al.*, 1987; Ratcliffe *et al.*, 1989). Exposure levels ranged from undetectable to 24 ppm with an average exposure levels of 6 ppm in one building and 11 ppm in the other. A statistically significant difference in mean sperm count was observed between exposed male workers and 39 unexposed male workers. Semen volume and pH, viability, motility, velocity, and morphology were not significantly different between the two groups. The

primary metabolite of EGEE, ethoxyacetic acid, was identified in the urine of exposed but not control workers. Both exposed and control subjects had significantly lower sperm counts than historical controls. Furthermore, members of both groups may have been exposed to other compounds including metals, solvents, heat, and vibration.

V. Effects of Animal Exposure

Sprague-Dawley rats (15/sex/group) and New Zealand white rabbits (10/sex/group) were exposed to 0, 25, 103, or 403 ppm EGEE by inhalation for 6 hours/days, 5 days/week, for 13 weeks (Barbee *et al.*, 1984). Animals were physically examined weekly and, at the end of the study, hematology, clinical chemistry, and histopathological examination were performed. No histopathological changes in the respiratory tract were found. Among rabbits, body weight was reduced in the high-dose group males and females. In the 25 ppm dose group, adrenal weight was reduced significantly among males, although this effect was not found to be dose-related. Among males in the high-dose group, testes weights were significantly reduced with a corresponding degenerative change to the seminiferous tubule epithelium. No effect on spermatogenic activity was found, however. Significant hematological effects observed at the high-dose included decreased hemoglobin, hematocrit, and erythrocyte count.

Teratologic effects in pregnant rats from the inhalation of EGEE were reported (Tinston *et al.*, 1983). The results of this study were presented in summary form (Doe, 1984). Wistar rats (24/group) were exposed to target concentrations of 0, 10, 50, or 250 ppm EGEE for 6 hours/day during gestational days 6-15 and the animals were sacrificed on day 21. Maternal toxicity was observed in the high-dose group with decreased hemoglobin, hematocrit, and mean corpuscular volume. Significant increases in preimplantation loss occurred in the 10 and 50 ppm dose groups, however the absence of this effect at 250 ppm indicated a poor dose-response, and because implantation occurred on the first day of exposure, the relatedness of the effect to exposure is in question. Post-implantation loss was also increased in the mid-dose group, however, no corresponding decrease in intrauterine death was observed in this group. Minor skeletal defects, particularly delayed ossification, were widely observed in the fetuses of mothers exposed to 250 ppm EGEE. Delayed ossification of the cervical vertebrae and sternebrae and the presence of extra ribs was significantly increased in both the 50 and 250 ppm dose groups.

Teratologic effects on pregnant rabbits from inhalation exposure to EGEE were also reported (Tinston *et al.*, 1983; also summarized by Doe, 1984). Dutch rabbits (24/group) were exposed to 0, 10, 50, or 175 ppm EGEE for 6 hours/day during gestational days 6-18, with sacrifice occurring on gestational day 29. There were no indications of maternal toxicity or litter effects. A statistically significant increase in minor defects and skeletal variants was found in fetuses in the 175 ppm dose group. Other slightly increased incidences of defects in the lower dose groups alone, including extra ribs and partial ossification of the vertebrae, were not considered treatment-related.

Behavioral teratogenic effects were examined in pregnant S-D rats (14 or 15/dose group) exposed to 0 or 100 ppm EGEE for 7 hours/day through gestational days 7-13 (early) or days 14-

20 (late) (Nelson *et al.*, 1981). No maternal toxicity was observed and fetal weights were unchanged, although mean gestational length was increased in rats exposed on gestational days 14-20. Six tests (ascent, rotorod, open field, activity wheel, avoidance conditioning, and operant conditioning) were selected to measure motor, sensory, and cognitive function at several stages of development. The offspring of the rats exposed during days 7-13 exhibited impaired performance on the rotorod test (a test of neuromuscular ability) and increased latency in an open field test (a test of exploratory activity) as compared to controls. The offspring of rats exposed during days 14-20 of gestation exhibited decreased activity on an activity wheel (a test of circadian activity). Also, avoidance conditioning revealed that these pups received shocks of a greater number and duration than controls. Neurochemical differences between the prenatally exposed and control pups were measured in newborns and in pups 21 days of age. In newborns from both EGEE-exposed groups, total brain norepinephrine was decreased. In 21-day old pups of both groups, norepinephrine and dopamine levels in the cerebrum were increased. Serotonin level was increased in the cerebrum of the late exposure group only. The authors concluded that there were behavioral and neurochemical alterations in offspring of rats following prenatal exposure to 100 ppm EGEE, however the study design was inadequate to detect gross teratologic anomalies. In a dose range-finding study, two sets of pregnant rats (3-4/group) were exposed during the gestational days 7-13 or 14-20 to 0, 200 (late group only), 300, 600, 900, or 1200 ppm EGEE for 7 hours/day. Increased fetal and pup mortality was observed in all groups exposed to EGEE.

Behavioral and neurochemical effects on the offspring of pregnant S-D rats exposed to 0 or 200 ppm EGEE on gestational days 7-13 were reported (Nelson *et al.*, 1982; Nelson *et al.*, 1982). Pregnancy duration was significantly increased in exposed dams. Significantly increased levels of norepinephrine and dopamine were observed in the 21-day old offspring of EGEE-exposed animals. Behavioral changes in pups of treated dams included decreased neuromotor ability and decreased activity.

An investigation into teratologic effects of EGEE was conducted by exposing pregnant rats and rabbits to EGEE by inhalation on gestational days 0-19 (Andrew *et al.*,). Rats (37/group) were exposed to 0, 202, or 767 ppm EGEE for 7 hours/day. All fetuses were resorbed and maternal weight gain was reduced in the high-dose group. In the mid-dose group, a decrease in fetal weight and size (crown-rump length) was observed. Minor skeletal defects and variants, cardiovascular defects were increased in the mid-dose group. Rabbits (29/group) were exposed to 0, 16, or 617 ppm EGEE for 4 hours/day. Maternal weight gain and food intake were decreased in exposed animals. The incidence of fetal resorptions was increased in both the mid- and high-dose group animals. Major cardiovascular defects and minor skeletal defects (extra ribs, delayed ossification) were significantly increased in the mid-dose group. Andrew *et al.* (1981) also examined reproductive effects by exposing female Wistar rats (37/group) to 1, 150, or 649 ppm EGEE 7 hours/day, 5 days/week for 3 weeks before mating with untreated males. No significant effects were observed.

VI. Derivation of U.S. EPA Reference Concentration (RfC)

Study	Barbee *et al.*, 1984
Study population	Rabbits
Exposure method	Discontinuous inhalation
Critical effects	Testicular degeneration and decreased hemoglobin
LOAEL	403 ppm
NOAEL	103 ppm
Exposure continuity	6 hr/day, 5 days/week
Exposure duration	13 weeks
Average experimental exposure	18.4 ppm (68 mg/m^3) for the NOAEL group
Human equivalent concentration	18.4 ppm (68 mg/m^3) for the NOAEL group (gas with systemic effects, based on RGDR = 1.0 using default assumption that lambda (a) = lambda (h))
Subchronic uncertainty factor	10
LOAEL uncertainty factor	1
Interspecies factor	3
Intraspecies factor	10
Cumulative uncertainty factor	300
Inhalation reference exposure level	0.06 ppm (60 ppb, 0.2 mg/m^3 , 200 µg/m^3)

Although reproductive toxicity has been reported in male workers occupationally exposed to EGEE (Clapp *et al.*, 1987; Ratcliffe *et al.*, 1989), potential confounding factors, particularly exposure to other compounds, make the study inadequate for the development of the reference exposure level.

The reproductive effects observed in the subchronic inhalation study of Barbee *et al.* (1984) were determined by the US EPA (U.S. EPA, 1990) to be the most sensitive endpoints for the development of the reference concentration (RfC). Reduced testes weight and testicular degeneration were found in rabbits exposed to EGEE at 403 ppm for 13 weeks. Changes in hematological parameters including decreased hemoglobin, hematocrit, and erythrocyte count were also observed at this dose. A gas:extrarespiratory effect ratio of 1.0 was used to calculate a human equivalency concentration (HEC) in the absence of information relating the effect in rabbits relative to humans.

The strengths of the inhalation REL include the availability of subchronic inhalation exposure data from a well-conducted study with histopathological analysis, and the observation of a NOAEL. Major areas of uncertainty are the lack of adequate human exposure data and the lack of chronic inhalation exposure studies.

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CHRONIC TOXICITY SUMMARY

ETHYLENE GLYCOL MONOETHYL ETHER ACETATE

(EGEEA; 1-acetoxy-2-ethoxyethane; 2-ethoxyethanol acetate; 2-ethoxyethyl acetate; acetic acid, 2-ethoxyethyl ester; beta-ethoxyethyl acetate; Cellosolve® acetate; ethoxy acetate; ethyl Cellosolve® acetate; Poly-solv® EE acetate; ethyl glycol acetate; oxitol acetate)

CAS Registry Number: 111-15-9

I. Chronic Toxicity Summary

Inhalation reference exposure level	**300 µg/m^3**
Critical effect(s)	Developmental toxicity and fetotoxicity in rabbits
Hazard index target(s)	Teratogenicity

II. Chemical Property Summary (HSDB, 1995)

Molecular formula	$C_6H_{12}O_3$
Molecular weight	132.16 g/mol
Description	Colorless liquid
Vapor pressure	2 mm Hg @ 20°C
Solubility	Soluble in ~6 parts water (229 g/l at 20°C); sol. in alcohol, ether, acetone; miscible with olive oil, aromatic hydrocarbons
Conversion factor	5.41 µg/m^3 per ppb at 25ºC

III. Major Uses and Sources

Ethylene glycol monoethyl ether acetate (EGEEA) is used in automobile lacquers where it retards "blushing" and evaporation and imparts a high gloss (HSDB, 1995). It is also used as a solvent for nitrocellulose, oils, and resins and as a component of varnish removers and wood stains. EGEEA is also used in the treatment of textiles and leather.

IV. Effects of Human Exposure

No studies relating exposure to EGEEA to adverse health effects in humans were located in the literature.

Ten male volunteers were exposed to EGEEA by inhalation. Five were exposed to 14, 28, and 50 mg EGEEA/m^3 and five to 28 mg/m^3 for 4 hours (Groeseneken *et al.*, 1987a). Twenty-two percent of the absorbed dose was eliminated in the urine as ethoxyacetic acid within 42 hours. In

another study, male volunteers exposed to EGEEA by inhalation under various conditions were found to eliminate some in the form of ethylene glycol monoethyl ether (EGEE) (Groeseneken *et al.*, 1987b).

V. Effects of Animal Exposure

Pregnant rabbits (24 or 25/group) were exposed to 0, 25, 100, or 400 ppm EGEEA by inhalation for 6 hours/day on gestational days 6-18 (Tinston *et al.*, 1983; reviewed in Doe, 1984). The animals were killed on gestational day 29. Maternal effects (decreased weight gain, decreased food consumption, decreased hemoglobin) were observed in the high-dose group. The number of rabbits with total fetal resorptions was increased in the 400 ppm dose group, accompanied by a decrease in weight in surviving fetuses. A reduction in average fetal weight was also observed at 100 ppm EGEEA, but this effect may relate to the increased litter size among dams in this dose group. Evidence of teratogenicity was observed in the 400 ppm dose group, with increased major malformations of the vertebral column. Both 400 and 100 ppm EGEEA were found to be fetotoxic as indicated by retarded ossification. No statistically significant effects were observed in the 25 ppm dose group, although a single case of a major defect (kidney agenesis) was observed in both the 25 and 400 ppm EGEEA dose groups.

Rats (10/sex/dose) and rabbits (2/sex/dose) were exposed for 4 hours/day, 5 days/week for 10 months to 0 or 200 ppm EGEEA (Truhaut *et al.*, 1979). Observation of body weight gain, hematology, clinical chemistry, and gross pathology revealed no toxic effects among treated animals. Among male rats and rabbits, “discrete lesions of tubular nephritis with clear degeneration of the epithelium with hyaline and granular tubular casts” were observed. Four hour exposure to 2000 ppm EGEEA resulted in transient hemoglobinuria and hematuria in rabbits (2/sex/dose), but not rats (10/sex/dose). No pathological lesions were observed following a 2 week observation period.

Dogs were exposed to 600 ppm EGEEA for 7 hours/day for 120 days (Carpenter *et al.*, 1956; Gingell *et al.*, 1982). Hematological, clinical chemistry, and histopathological examination revealed no adverse effects.

Pregnant rats and rabbits (24/group) were exposed to nominal concentrations of 0, 50, 100, 200 or 300 ppm EGEEA by inhalation during gestational days 6-15 and sacrificed on gestational day 21 (Union Carbide Corporation, 1984). Maternal effects in rats included increased absolute liver weights (all treated groups); increased relative liver weights, and decreased RBC count, hemoglobin, hematocrit, and RBC size (all but low-dose group); decreased food consumption, increased white blood cell count, and decreased platelet count (200 and 300 ppm groups). An increase in the number of non-viable implantations per litter was observed at 300 ppm and decreased average fetal body weight per litter was observed at 200 and 300 ppm EGEEA. Visceral and skeletal malformations were widely observed at both 200 and 300 ppm EGEEA. Among rabbits, maternal effects included decreased platelets (100, 200, and 300 ppm); decreased weight gain, decreased gravid uterine weight, increased number of dams with non-viable implants, and increased number of non-viable implants per litter (200 and 300 ppm); increased

occult blood, increased mean corpuscular volume, decreased corpora lutea/litter and increased early resorptions/litter (300 ppm). Visceral and skeletal malformations were observed in the 100, 200, and 300 ppm EGEEA dose groups.

Pregnant rats were exposed to 0, 130, 390, or 600 ppm EGEEA for 7 hours/day on gestational days 7-15 (Nelson *et al.*, 1984). Dams were sacrificed on day 20. Complete resorption of litters was observed at 600 ppm. Skeletal and cardiovascular defects and decreased fetal weight and fetal resorptions were observed at 390 ppm EGEEA. Reduced fetal weights were also observed at 130 ppm EGEEA.

Ethylene glycol monoethyl ether acetate (0.35 ml = 2.6 mmole/treatment) or water was applied to the shaved interscapular skin of pregnant rats four times daily on days 7 to 16 gestation (Hardin *et al.*, 1984). EGEEA treated rats showed reduced body weight (from litter resorption) and significantly fewer live fetuses per litter. Litters from treated dams also showed significantly increased visceral malformations and skeletal variations.

VI. Derivation of Chronic Reference Exposure Level (REL)

Study	Tinston *et al.*, 1983
Study population	Rabbits
Exposure method	Discontinuous inhalation exposure
Critical effects	Fetotoxicity
LOAEL	100 ppm
NOAEL	25 ppm
Exposure continuity	6 hours/day, 7 days/week
Exposure duration	13 days
Average experimental exposure	6.2 ppm for NOAEL group (25 x 6/24)
LOAEL uncertainty factor	1
Subchronic uncertainty factor	1
Interspecies factor	10
Intraspecies factor	10
Cumulative uncertainty factor	100
Inhalation reference exposure level	0.06 ppm (60 ppb, 0.03 mg/m^3, 300 $\mu g/m^3$)

A review of the literature on the toxicity of EGEEA indicates that the most sensitive endpoint of toxicity is that seen in experimental animals showing developmental effects from inhalation exposure during pregnancy. There are no adequate data associating exposures in humans with toxic effects for the development of a chronic reference exposure level. Separate studies in animals have demonstrated developmental toxicity. Reduced fetal weights were observed in rats exposed to 130 ppm EGEEA on gestational days 7-15 (Nelson *et al.*, 1984). Skeletal and cardiovascular defects were observed at the next higher dose of 390 ppm EGEEA, and all litters were resorbed in the high-dose group. Visceral and skeletal defects were observed in all but the low-dose group (50 ppm EGEEA) in the litters of rabbit dams exposed to EGEEA on gestational days 6-15 (Union Carbide Corporation, 1984). Fetotoxicity, as indicated by retarded bone

development, was observed in all but the low-dose group (25 ppm EGEEA) in the litters of rabbit dams exposed on gestational days 6-18 (Tinston *et al.*, 1983). The lowest dose levels showing developmental toxicity are those reported by Union Carbide Corporation (1984) and Tinston *et al.* (1983), with 100 ppm EGEEA showing developmental defects in the offspring of exposed dams. Since only the Tinston *et al.* (1983) study also showed an exposure level without effect (a NOAEL), this study has been selected for the development of the chronic REL.

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CHRONIC TOXICITY SUMMARY

ETHYLENE GLYCOL MONOMETHYL ETHER

(EGME; 2-methoxyethanol; 1-hydroxy-2-methoxyethane; methyl cellosolve)

CAS Registry Number: 109-86-4

I. Chronic Toxicity Summary

Inhalation reference exposure level	**20 µg/m^3** (U.S. EPA RfC) This document summarizes the evaluation of non-cancer health effects by U.S. EPA for the RfC.
Critical effect(s)	Testicular toxicity in rabbits
Hazard index target(s)	Reproductive system

II. Physical and Chemical Properties (HSDB, 1995)

Molecular formula	$C_3H_8O_2$
Molecular weight	76.09
Description	Colorless liquid
Specific gravity	0.965 @ 20° C
Boiling point	125° C
Melting point	-85.1° C
Vapor pressure	6.2 mm Hg @ 20° C
Solubility	Miscible with water, alcohol, benzene, ether, acetone
Conversion factor	1 ppm = 3.1 mg/m^3 @ 25°C

III. Major Uses and Sources

Ethylene glycol monomethyl ether (EGME) is used as a solvent for cellulose acetate and resins (HSDB, 1995). It is also used in dyeing leather and in the manufacture of photographic film. EGME is used as an anti-freeze in jet fuels. Quick drying varnishes, enamels, nail polishes, and wood stains may also contain EGME.

IV. Effects of Human Exposure

Decreased testicular size was reported in workers exposed to an 8-hour TWA concentration of 0.42 ppm EGME or less (Cook *et al.*, 1982).

Reversible neurological symptoms (apathy, fatigue, decreased appetite) and macrocytic anemia were observed in a worker following occupational dermal and inhalation exposure to an average concentration of 35 ppm EGME for 1-1.5 years (Cohen, 1984). The worker was also exposed to methyl ethyl ketone and propylene glycol monomethyl ether at concentrations of 1-5 ppm and 4.2-12.8 ppm, respectively.

Hematologic effects were also reported in three women employed in a factory working with glue consisting of 70% acetone and 30% EGME (Larese *et al.*, 1992). The women exhibited abnormally low white blood cell counts, relative lymphocytosis and macrocytosis. These hematological parameters returned to normal following cessation of exposure.

Older case reports support findings of neurological and hematological toxicity following occupational exposure to EGME (Greenburg *et al.*, 1938; Zavon, 1963; Parsons and Parsons, 1938).

V. Effects of Animal Exposure

A concentration dependent decrease in testes weight was observed in male rabbits exposed to 30, 100, or 300 ppm EGME 6 hours per day, 5 days per week for 13 weeks (Miller *et al.*, 1983). Degenerative changes in the germinal epithelium was observed in male rabbits of all exposed groups. Two of five male rabbits exposed to 300 ppm EGME died during the course of the study. Female rabbits were also exposed; two of five female rabbits exposed to 100 or 300 ppm EGME died during the course of the study. Reduced body weight gain, pancytopenia (abnormal depression of all the cellular elements of the blood), and thymic atrophy were observed in rabbits of both sexes exposed to 300 ppm EGME. No effects on the reproductive organs of the female rabbits were observed.

In the same study (Miller *et al.*, 1983) male and female rats were exposed to 30, 100, or 300 ppm EGME 6 hours per day, 5 days per week for 13 weeks. Moderate to severe degeneration of the germinal epithelium and seminiferous tubules was observed in male rats exposed to 300 ppm EGME. A significant decrease in body weight was observed in male rats exposed to 300 ppm and in female rats exposed to concentrations of EGME of 100 ppm or greater. Pancytopenia, lymphoid tissue atrophy, and decreased liver weights were observed in animals of both sexes exposed to the highest concentration. Also in the highest exposure group, mean values for total serum protein, albumin and globulins were lower than control values.

More recent data point to the immune system as a key endpoint of EGME toxicity. A statistically significant dose-related decrease in thymus weight was observed both in male rats administered drinking water containing 2000 and 6000 ppm EGME (161 or 486 mg/kg/day) and in female rats administered drinking water containing 1600 and 4800 ppm EGME (200 or 531 mg/kg/day) for 21 days (Exon *et al.*, 1991). Histopathological examination revealed thymic atrophy and loss of demarcation between the cortex and medulla. Decreased spleen cell numbers were observed in female rats at both dose levels and male rats at the high dose level. Male rats in the high dose

group exhibited a statistically significant decrease in body weight gain. Testicular effects were also observed in exposed male rats.

Pregnant mice were exposed to 100, 150, or 200 mg/kg/day EGME on days 10-17 of gestation (Holladay *et al.*, 1994). Thymic atrophy and inhibition of fetal thymocyte maturation were observed in EGME-treated offspring examined on day 18 of gestation. Also, the ability of the EGME-treated fetal mouse liver cells to repopulate the spleen of irradiated mice was significantly impaired as compared to that of control fetal mouse liver cells.

VI. Derivation of U.S. EPA RfC

Study	Miller *et al.*, 1983; U.S. EPA (1995)
Study population	Rats and rabbits
Exposure method	Inhalation (0, 30, 100, or 300 ppm)
Critical effects	Decreased testes weight and degenerative changes in the testicular germinal epithelium.
LOAEL	100 ppm
NOAEL	30 ppm
Exposure continuity	6 hours/day, 5 days/week
Average experimental exposure	5.4 ppm for NOAEL group
Human equivalent concentration	5.4 ppm for NOAEL group (gas with systemic effects, based on RGDR = 1.0 using default assumption that lambda (a) = lambda (h))
Exposure duration	13 weeks
LOAEL uncertainty factor	1
Subchronic uncertainty factor	10
Interspecies uncertainty factor	3
Intraspecies uncertainty factor	10
Modifying factor	3
Cumulative uncertainty factors	1,000
Inhalation reference exposure level	0.005 ppm (5 ppb; 0.02 mg/m^3; 20 $\mu g/m^3$)

The strengths of the inhalation REL include the availability of subchronic inhalation exposure data from a well-conducted study with histopathological analysis and the observation of a NOAEL. Major areas of uncertainty are the lack of adequate human exposure data, and the lack of chronic inhalation exposure studies.

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CHRONIC TOXICITY SUMMARY

ETHYLENE GLYCOL MONOMETHYL ETHER ACETATE

(EGMEA; 2-methoxyethanol acetate; 2-methoxyethylester acetic acid; methyl glycol acetate; methyl Cellosolve® acetate)

CAS Registry Number: 110-49-6

I. Chronic Toxicity Summary

Inhalation reference exposure level	**90 μg/m^3**
Critical effect(s)	Reproductive (testicular) toxicity in rabbits (EGME)
Hazard index target(s)	Reproductive system

II. Chemical Property Summary (HSDB, 1995)

Molecular formula	$C_5H_{10}O_3$
Molecular weight	118.3 g/mol
Description	Colorless liquid
Vapor pressure	2 mm Hg @ 20°C
Solubility	Miscible with water, organic solvents, oils
Conversion factor	4.83 μg/m^3 per ppb at 25ºC

III. Major Uses and Sources

Ethylene glycol monomethyl ether acetate (EGMEA) is used as a solvent for nitrocellulose, cellulose acetate, and various other gums, resins, waxes, and oils (HSDB, 1995). It is also used in textile printing, photographic films, lacquers, and silk-screening inks.

IV. Effects of Human Exposure

Developmental defects have been described in the offspring of a mother who was occupationally exposed to EGMEA during pregnancy (Bolt and Golka, 1990). The mother was exposed during pregnancy by skin absorption and inhalation for approximately 1-4 hours/day to 1-2 liters of EGMEA. Her first child was born with congenital hypospadia, chordee, micropenis, and scrotum bifida and her second child (3 years later) was born with chordee, cryptorchidism, penile hypospadia and scrotum bifida. Both children had normal karyotypes. No estimates of exposure were made.

A single case report described allergic dermatitis which may have developed from contact with EGMEA (Jordan and Dahl, 1971). A 58-year-old woman developed dermatitis on the nose possibly from contact with EGMEA on her eyeglasses. Ethylene glycol monoethyl ether acetate (EGEEA) was also present.

V. Effects of Animal Exposure

Cats, rabbits, guinea pigs, and mice were repeatedly exposed by inhalation for 8 hours daily to 500 and 1000 ppm EGMEA (Gross, 1943; as described by Gingell *et al.*, 1982). This exposure regimen was fatal to cats at 500 ppm EGMEA. Death occurred after the animals showed slight narcosis. Similarly, exposure to 1000 ppm EGMEA produced deaths among rabbits, guinea pigs, and mice within a few days. Kidney toxicity was observed in animals in both dose groups. Repeated 4- and 6-hour exposure of cats to 200 ppm EGMEA resulted in decreased "blood pigments" and red blood cell counts.

The toxic effects of EGMEA were examined in male mice treated by gastric intubation 5 days/week for 5 weeks with 0, 62.5, 125, 250, 500, 1000, or 2000 mg EGMEA/kg/day (Nagano *et al.*, 1984). Dose-related testicular atrophy was observed at doses above 250 mg EGMEA/kg/day. Decreased white blood cell counts were observed in all EGMEA-exposed groups.

EGMEA was readily converted in vitro to ethylene glycol monomethyl ether (EGME) by the nasal mucosal carboxylesterases of mice and rabbits (Stott and McKenna, 1985). The enzyme activity in the nasal mucosa was equal to that of the liver and greater than that of the kidney and lung.

A concentration dependent decrease in testes weight was observed in male rabbits exposed to 30, 100, or 300 ppm ethylene glycol monomethyl ether (EGME) 6 hours/day, 5 days/week for 13 weeks (Miller *et al.*, 1983). Degenerative changes in the germinal epithelium was observed in male rabbits of all exposed groups. Two of five male rabbits exposed to 300 ppm EGME died during the course of the study. Female rabbits were also exposed; two of five female rabbits exposed to 100 or 300 ppm EGME died during the course of the study. Reduced body weight gain, pancytopenia (abnormal depression of all the cellular elements of the blood), and thymic atrophy were observed in rabbits of both sexes exposed to 300 ppm EGME. No effects on the reproductive organs of the female rabbits were observed.

In the same study male and female rats were exposed to 30, 100, or 300 ppm EGME 6 hrs/day, 5 days/week for 13 weeks. Moderate to severe degeneration of the germinal epithelium and seminiferous tubules was observed in male rats exposed to 300 ppm EGME. A significant decrease in body weight was observed in male rats exposed to 300 ppm and in female rats exposed to concentrations of EGME of 100 ppm or greater. Pancytopenia, lymphoid tissue atrophy, and decreased liver weights were observed in animals of both sexes exposed to the highest concentration. Also in the highest exposure group, mean values for total serum protein, albumin and globulins were lower than control values.

VI. Derivation of Chronic Reference Exposure Level (REL)

(Based on USEPA RfC for EGME)

Study	Miller *et al.*, 1983 (see below)
Study population	Rabbits
Exposure method	Discontinuous inhalation exposure (0, 30, 100, or 300 ppm EGME)
Critical effects	Testicular effects
LOAEL	100 ppm EGME
NOAEL	30 ppm EGME
Exposure continuity	6 hr/day, 5 days/week
Exposure duration	13 weeks
Average experimental exposure	5.4 ppm EGME for NOAEL group (30 x 6/24 x 5/7)
Human equivalent concentration	5.4 ppm EGME for NOAEL group (gas with systemic effects, based on RGDR = 1.0 using default assumption that lambda (a) = lambda (h))
LOAEL uncertainty factor	1
Subchronic uncertainty factor	10
Interspecies factor	3
Intraspecies factor	10
Cumulative uncertainty factor	300
Inhalation reference exposure level	0.02 ppm (20 ppb, 0.06 mg/m^3, 60 µg/m^3) EGME 90 µg/m^3 EGMEA (60 x MW_{EGMEA} / MW_{EGME})

Data relating specific EGMEA exposure levels to toxicity in humans are not available for the development of a chronic REL. Data from experimental animals indicates that EGMEA is toxic to the hematopoietic and reproductive systems (Gross, 1943; Nagano *et al.*, 1984), however good, quantitative data relating chronic exposure to toxicity is lacking. Because of evidence that EGMEA is readily converted to EGME by several organ systems (Stott and McKenna, 1985) and since the scant data on EGMEA toxicity in animals indicate that the spectrum of toxicity of the two compounds is similar, the chronic REL was derived based upon the assumption of equimolar toxicity of EGMEA and EGME. The reference concentration for EGME reported by U.S. EPA was used to derive the EGMEA REL.

The strengths of the inhalation REL include the availability of subchronic inhalation exposure data from a well-conducted study, and the observation of a NOAEL. Major areas of uncertainty are the assumption that EGMEA toxicity is comparable to that of EGME, the lack of adequate human exposure data, and the lack of chronic inhalation exposure studies.

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CHRONIC TOXICITY SUMMARY

ETHYLENE OXIDE

(Oxirane, dimethylene oxide, epoxyethane)

CAS Registry Number: 75-21-8

I. Chronic Toxicity Summary:

Inhalation reference exposure level	**5 μg/m^3**
Critical effect(s)	Hematologic changes in humans
Hazard index target(s)	Circulatory system; respiratory system; nervous system

II. Physical and Chemical Properties (HSDB, 1995)

Description	Colorless gas
Molecular formula	C_2H_4O
Molecular weight	44.06
Specific gravity	0.8222 @ 10ºC
Boiling point	10.7ºC
Vapor pressure	1095 torr @ 20ºC
Conversion factor	1 ppm = 1.80 mg/m^3

III. Major Uses or Sources

The majority of all ethylene oxide (EtO) produced is used as a chemical intermediate in the production of various compounds including ethylene glycol, glycol ethers, and non-ionic surfactants (ATSDR, 1990). EtO is also used as a fumigant for food and cosmetics, and in hospital sterilization of surgical equipment and heat sensitive materials such as plastics.

IV. Effects of Human Exposure

Ten hospital sterilizer workers were matched with controls and examined for physical and neuropsychological health (Estrin *et al.*, 1990). The workers had operated sterilizers using 12% EtO and 88% Freon for an average of 5 years (range 0.5-10 years). Regular monitoring of workroom air had not been done. Measurements at the time of the study indicated concentrations of 15 ppm EtO or less. However, a second measurement showed an air concentration of 250 ppm EtO. A significantly greater percent of exposed workers exhibited a bilateral reflex reduction in the ankle compared to controls. Nerve conduction tests did not identify significant differences between control and exposed workers, but a highly significant reduction ($p = 0.009$)

in finger tapping speed was observed in exposed workers. The exposed group also performed more poorly on tests of spatial and visual abilities, and on tests of visual motor function.

Cognitive impairment and personality dysfunction were observed more frequently in hospital workers chronically exposed to EtO, compared to a control group (Klees *et al.*, 1990). A group of 22 hospital workers who had been exposed to an 8-hour TWA of 4.7 ppm EtO for a mean of 6.13 years (range 1-11 years) were matched with 24 control subjects. Worker neuropsychological function was classified as normal or impaired on the basis of the questionnaires and neuropsychological tests by 2 clinical psychologists unaware of exposure status. (If the classification of the two clinicians did not agree, the subject was classified as "disagreement".) Compared to controls, exposed subjects were significantly more frequently classified as impaired.

Recent studies have identified hemoglobin adducts, sister chromatid exchanges and other hematological effects as indicators of ethylene oxide exposure (Ribeiro *et al.*, 1994; Sarto *et al.*, 1991;). However, a recent study of 68 female workers from 9 US and one Mexico hospital not only reports biological indicators of ethylene oxide exposure, but also provides a complete blood count with differential (Schulte *et al.*, 1995). In this study, the workers were classified as low- or high-exposure based on a mean 8-hour time weighted average of 0.08 or 0.17 ppm EtO. The mean length of employment for workers from US hospitals was 5.5 and 10 years for low- and high-exposure workers respectively. The mean length in low- and high-exposure workers from the Mexico hospital was 5.9 and 4.2 years, respectively. In workers from US hospitals only, statistically significant decreases in hematocrit and hemoglobin were observed in high-exposure workers compared to low-exposure workers. Also, a statistically significant increase in lymphocytes and a significant decrease in neutrophils were observed in high-exposure workers compared to controls. In the workers from the Mexico hospital, a significant relationship of EtO exposure and elevated neutrophil count was observed using regression.

V. Effects of Animal Exposure

A 2-year inhalation bioassay exposed groups of 80 male rats to 0, 50, or 100 ppm EtO 7 hours per day, 5 days per week for 104 weeks (Lynch *et al.*, 1984). Mean body weights were significantly lower and mortality was significantly higher in both exposure groups. Inflammatory lesions of the lung, nasal cavity, trachea and inner ear were observed more frequently in EtO exposed rats. Skeletal muscle myopathy, consisting of atrophy and degeneration of skeletal muscle fibers, was observed more frequently in rats exposed to 100 ppm EtO compared to controls. Neoplastic changes were also observed in EtO exposed rats.

Mice (30 per sex) were exposed to 0, 10, 50, 100, or 250 ppm EtO for 6 hours per day, 5 days per week, for 10 weeks (males) or 11 weeks (females) (Snellings *et al.*, 1984). Neuromuscular screening was conducted, and samples of urine and blood were collected. A significantly greater percent of exposed mice exhibited abnormal posture during gait and reduced locomotor activity. A dose-response was observed for these effects, with significant changes at 50 ppm and greater. An abnormal righting reflex was observed in a significantly greater percent of mice exposed to

100 ppm and above. Reduced or absent toe and tail pinch reflexes were observed in a significantly greater percent of mice exposed to 250 ppm EtO. Hematological changes observed in mice exposed to 250 ppm include slight, yet significant, decreases in red blood cell count, packed cell volume, and hemoglobin concentration. Absolute and relative spleen weights were significantly decreased in female mice exposed to 100 and 250 ppm and in male mice exposed to 250 ppm EtO. A significant increase in relative liver weight was observed in female mice exposed to 250 ppm EtO. Male mice exhibited a significant decrease in body weight at 10, 50, and 250 ppm and a significant decrease in absolute testes weights at 50, 100, or 250 ppm EtO. This study indicates a subchronic NOAEL for neurological effects of 10 ppm EtO.

In a study of the testicular effects of EtO, male rats were exposed to 500 ppm EtO 6 hours per day, 3 days per week for 2, 4, 6, or 13 weeks (Kaido *et al.*, 1992). An awkward gait was observed in rats after 6-9 weeks of exposure. Although no significant changes in body weight were observed, a statistically significant dose-related decrease in testes weight was observed at 4, 6, and 13 weeks of exposure. Progressive degeneration and loss of germ cells were also observed during the 13 week exposure. While severe loss of germ cells and marked morphological changes in remaining germ cells were observed at 6 weeks of exposure, some intact spermatids were observed at 13 weeks of exposure. This suggests that recovery of spermatogenesis occurred.

VI. Derivation of Chronic Reference Exposure Level (REL)

Study	Schulte *et al.*, 1995
Study population	28 or 10 Female U.S. hospital workers for the high and low exposure categories, respectively
Exposure method	Occupational 8-hour/day inhalation
Critical effects	Reduced hematocrit and hemoglobin; elevated lymphocyte count; reduced neutrophil count; presence of hemoglobin adducts, micronuclei and SCEs in the blood.
LOAEL	0.17 ppm
NOAEL	0.08 ppm
Exposure continuity	8 hours/day (10 m^3/day occupational inhalation rate), 5 days/week
Exposure duration	6.8 years (mean length of employment) 5.5 years for NOAEL group 10 years for LOAEL group
Average experimental exposure	0.029 ppm for NOAEL group (0.08 x 10/20 x 5/7)
Human equivalent concentration	0.029 ppm
LOAEL uncertainty factor	1
Subchronic uncertainty factor	1
Interspecies uncertainty factor	1

Intraspecies uncertainty factor	10
Cumulative uncertainty factor	10
Inhalation reference exposure level	0.003 ppm (3 ppb; 0.005 mg/m^3; 5 $\mu g/m^3$)

The strengths of the inhalation REL include the use of human exposure measurements taken from workers who had been working with EtO over a period of years and the observation of a NOAEL. Major areas of uncertainty are the uncertainty in estimating exposure, the potential variability in exposure concentration, and the lack of developmental toxicity studies. Schulte *et al.* (1995) did not give adequate information on their EtO analyses and calculations in their report.

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CHRONIC TOXICITY SUMMARY

ETHYLENETHIOUREA

(ETU, 2-Mercaptoimidazoline, 4,5-Dihydro-2-mercaptoimidazole, 2-Imidazoline-2-thiol)

CAS Registry Number: 96-45-7

I. Chronic Toxicity Summary

Inhalation reference exposure level	**3 μg/m^3**
Oral reference exposure level	**0.01 mg/kg-day**
Critical effects(s)	Increased thyroid weight in rats
Hazard index target(s)	Endocrine system; alimentary system

II. Chemical Property Summary (IARC, 1974; HSDB, 1997; Howard & Meyland, 1997)

Molecular formula	$C_3H_6N_2S$
Molecular weight	102.17
Description	White crystals, mp = 200-203^{o}C
Vapor pressure	0.005 mmHg@25 ^{o}C
Boiling point	347 ^{o}C
Solubility	Moderately soluble in methanol, ethanol, ethylene glycol, pyridine. Slightly soluble at room temperature in acetic acid and naphtha. Insoluble in acetone, ether, chloroform, benzene, ligroin. In water, solubility is 2 percent at 30^{o}C, 9 percent at 60^{o}C, and 44 percent at 90^{o}C.
Conversion factor	4.18 μg/m^3 per ppb at 25^{o}C

III. Major Uses and Source (IARC, 1974; Lentza-Rizos, 1990; HSDB, 1997)

There are two major sources of exposure to ethylenethiourea (ETU): manufacturing and agriculturally related activities. During the manufacture of neoprene rubber, ETU serves as an accelerator, and exposure can occur during production, bagging, or delivery of ETU into the reaction vessel. ETU is also an intermediate in the syntheses of insecticides, fungicides, dyes, and pharmaceuticals. During the production of the fungicidal ethylenebisdithiocarbamates (EBDCs), the EBDCs may chemically decompose to ETU. Decomposition into ETU also occurs in the fields after application of the EBDCs, and during post-harvesting and food processing activities. The level of ETU in the final processed product depends on many factors including EBDC level, interval between application and harvest, and temperature during processing.

IV. Effects of Human Exposure

Hemoglobin-ETU (Hb-ETU) adducts were measured in the blood of workers in a factory where EBDC was manufactured (Pastorelli *et al.*, 1995). Among the 15 exposed workers, 6 exhibited values greater than the limit of detection (< 0.5 pmol/mg Hb). Workers (n=8) in the same factory who were not exposed to EBDC did not exhibit Hb-ETU adducts above the limit of detection. The wide range of Hb-ETU adducts among the exposed 15 workers (0.5 - 1.42 pmol / mg Hb) could be due to differences in metabolism (adduct formation requires metabolic activation) and / or varying degrees of use of protective clothing. Confounding factors of age, smoking habits and alcohol did not explain the results. Although the data show that exposure to the EBDCs during the manufacturing phase leads to exposure to ETU, the small sample number prevents more detailed analysis.

The ETU in the Hb-ETU adducts of the workers could only be released from the protein by acid hydrolysis, and not by organic solvent extraction. Hence the Hb-ETU adducts represent covalently bound products. The reaction between ETU and isolated human blood required the presence of liver (rat) microsomal fraction and reducing equivalents in the form of reduced nicotinamide adenine dinucleotide phosphate (NADPH) (Pastorelli *et al.*, 1995). This requirement suggests a role for the cytochrome P450-dependent monooxygenase enzymes (P450) or the flavin monooxygenase enzymes (FMO). The role of each of these enzyme systems is discussed later in the narrative.

Thyroid function was measured in workers exposed to ETU in manufacturing plants (Smith, 1984). The work activities included ETU production, bagging the final powder (factory 1) and opening sacks of ETU powder and adding the contents to a reaction vessel for further processing (factory 2). Although air samples were taken at various times, changes in workplace hygiene have resulted in decreased exposure of the workers to dust and airborne ETU. Hence, an accurate dose-response analysis is not possible. The exposure duration among the workers was 5 - 20 years. Thyroid function was assessed by levels of thyroxine (T_4), thyroid stimulating hormone (TSH), and thyroid binding globulin (TBG) in venous blood. The workers were divided into controls (n=40 factory workers without exposure to ETU), processing workers (n=23) and mixers (n=22). Compared to controls, T_4 levels were decreased among the mixers, and not among the processors. TSH levels among the controls and processors were within normal limits. One mixer who exhibited very high TSH levels was found to have premyxoedema, a condition leading to a severe form of hypothyroidism (Anderson and Anderson, 1994). TBG levels and the ratio of thyroxine to TBG were within normal limits (not specified) among the three groups. The results suggest exposure of the mixers to airborne ETU may lead to altered thyroid function. The results also indicate that inhalation exposure to ETU can result in the transport of ETU from the lung to other tissues.

Agricultural workers, who use EBDC formulations, are exposed to ETU from two sources: environmental breakdown of EBDC formulations (Lentza-Rizos, 1990) and metabolic breakdown of EBDCs in the body (Pastorelli *et al.*, 1995). Among potato farm workers, exposed to EBDCs during mixing and application activities, the mean breathing zone level of ETU was

843 ng/m^3 during one 4-hour application interval (14 farmers). The authors assumed a 100 percent absorption of ETU and calculated an uptake of about 5 µg ETU. Measured urinary levels, however, ranged from < 2 to 47 µg ETU over a 22-day interval after the end of exposure, and suggested an uptake of > 100 percent of the estimated inhaled ETU by some subjects. This discrepancy may by due additional exposures, for example, unprotected dermal and post-application exposures to the EBDC formulations. These additional exposures may also help to explain the large variability in urinary ETU among the 14 workers and the long estimated half-life of about 100 hours.

V. Effects of Animal Exposure

Ingested ETU is rapidly excreted into the urine (WHO, 1988). In rats, 82-99 percent of ingested ETU was found in the urine within 48 hours, and in monkeys, 55 percent of the administered dose was observed in urinary products at the end of the same interval. The following biological half-lives for ETU (route of administration not specified) have been reported: 5 hours (mouse), 9-10 hours (rat), and 28 hours (monkey) (WHO, 1988).

Rats exposed one time to ETU at doses of 0, 62.5, 125, 250, or 500 mg/kg exhibited Hb-ETU adducts at 24-hours post-exposure (Pastorelli *et al.*, 1995). The ETU was covalently bound to the Hb and the levels of Hb-ETU increased between 62.5 and 250 mg/kg. Incubations with isolated rat Hb showed that the binding was dependent on enzyme activity and protein thiols (cysteine) were probably the site for adduction.

ETU metabolism in animals is complex and leads to many products, although unmetabolized ETU is usually present (WHO, 1988). The proportion of ETU excreted unmetabolized depends on the ingested dose. In rats ingesting 11, 18, or 23 mg ETU/kg (drinking water), urinary ETU represented 25, 36, or 49 percent, respectively, of the ingested amount over a 28-day interval (Kurttio *et al.*, 1991). These results suggest ETU, at high doses, may inhibit its own metabolism. In rats receiving one dose of 4 mg/kg ETU (labeled in the 4- and 5- positions of the imidazole ring) in drinking water, unmetabolized ETU represented 63 percent of the urinary products over a 24-hr interval (Iverson, 1980). Three other metabolites excreted into the urine were imidazoline (1.9 percent), imidazolone (4.9 percent), and ethyleneurea (18.3 percent). An unknown metabolite(s) constituted 12.3 percent of the urinary metabolites.

The metabolite profile of ETU, administered intravenously (iv) to cats, is qualitatively and quantitatively different from the profile observed in rats exposed by ingestion (Iverson *et al.*, 1980). Unmetabolized ETU constituted 28 percent of the products excreted in the urine, and a different metabolite, S-methyl ETU (64.3 percent) was observed. Ethyleneurea (3.5 percent) and an unknown product (4.2 percent) were also present in the urine. Although the data suggest the metabolism of ETU may be species specific, they cannot exclude a different pathway due to the presence of liver metabolism in the rat, consistent with a first-pass effect. In the cat, the half-life of [^{14} C] ETU following the single iv dose was 3.5 hours, similar to the half-life in rat of 5.6 hours (quoted in Iverson *et al.*, 1977).

In rats, the initial metabolism of ETU is under the catalytic regulation of the microsomal P450 and FMO enzyme systems (Decker and Doerge, 1991). Each enzyme system requires reduced nicotinamide dinucleotide phosphate (NADPH) and oxygen. A product of either enzyme-catalyzed reaction is the corresponding sulfenic acid, which may be responsible for the NADPH-dependent ETU inhibition of P450 and FMO activities, including the metabolism of ETU. The ETU-dependent inhibition of its own metabolism could explain, in part, the observation by Kurttio *et al.* (1991), that increased urinary levels of unmetabolized ETU occurred as the dose of ETU was also increased.

Non-cancer toxicity endpoints were evaluated in rats and mice exposed to dietary ETU for 13-weeks and 2-years (Chhabra *et al.*, 1992). The purpose of the 13-wk study was to establish the doses for the 2-year study which included an evaluation of carcinogenicity. The lifetime study included a perinatal exposure to determine if the prior exposure made the rats and mice more sensitive to the effects of dietary ETU. Because the mice received higher doses of ETU and the results were similar to those obtained with the rats, the discussion will be limited to the rat data.

In the 13-week study, rats received 0, 60, 125, 250, 500, or 750 ppm ETU in the diet. The rats were 8-9 weeks of age at the start of the 13 week exposure. Among the male (M) and female (F) rats, diffuse follicular cell hyperplasia of the thyroid gland was observed at 60 ppm. Liver centrilobular cytomegaly was observed at 750 ppm (M+F). Pituitary gland cellular vacuolization occurred at 750 ppm (F) and 250 ppm (M). A subchronic lowest observed adverse effect level (LOAEL) of 60 ppm is suggested by the data.

To determine if prior exposure to ETU would make the rats more sensitive to lifetime ETU exposure, Chhabra et al (1992) exposed rats to ETU *in utero* and immediately following birth after which they were exposed in the 2-year study. According to the exposure regimen, females were exposed to dietary ETU (0, 9, 30, or 90 ppm) for 1-week prior to mating, during gestation, and during nursing. At post-natal week-4, the pups were weaned and exposed at the maternal level until the pups were 8-weeks old. Then they were entered into the chronic study at dietary ETU levels of 0, 25, 83, or 250 ppm. Observations were made at 9-months and 2-years. To distinguish the prior exposure (0, 9- to 90 ppm ETU) from the chronic exposure regimen (0, 25- to 250 ppm), the former was labeled by the authors as perinatal exposure (F_0), the latter as adult exposure (F_1), and the combined exposures as perinatal:adult (F_0:F_1). Although the F_0 and F_1 administered dose ranges are each in a group of 4, the combined F_0:F_1 exposures are not matches, i.e., for F_0 = 0, 9, 30, or 90 ppm and F_1 = 0, 25, 83, or 250 ppm, the F_0:F_1 exposures are different combinations as described in Chhabra *et al.* (1992). The doses were determined by the results of a 13-week subchronic study during which exposure to the adult females did not result in decreased implantations, numbers of fetuses per litter, live births, or fetal weights, but did result in decreased survival among the pups by postnatal day 4 at 250 ppm, decreased body weight gain among male pups at 83 ppm ETU, and diffuse follicular cell hyperplasia at 25 ppm (M) and 83 ppm (F) (data not shown).

Among the adult rats, chronically exposed for 2-years, with <u>no perinatal</u> exposure, the dietary doses were 0, 83, and 250 ppm ETU. Among these rats, those sacrificed at 9 months, exhibited increased incidences of thyroid follicular cell hyperplasia (M+F), decreased T_4 levels (M+F), and

increased thyroid stimulating hormone (TSH) levels (F) at 83 ppm. A chronic LOAEL of 83 ppm, based on thyroid follicular cell hyperplasia and thyroid function, is suggested. Rats exposed for 2 years exhibited increased incidence of thyroid follicular cell hyperplasia (M+F assumed from the narrative although not explicitly stated), decreased triiodothryonine (T_3) (F) and T_4 levels (M), and increased TSH levels (M+F) at 83 ppm ETU. A chronic LOAEL of 83 ppm, based on thyroid follicular cell hyperplasia and thyroid function is suggested by this study. It is important to understand that the lowest adult only dose of ETU in the Chhabra *et al.* (1992) study is 83 ppm.

Among the 9-month exposed rats who exhibited decreased T_3 and T_4 levels and increased TSH levels, perinatal exposure appeared to influence the outcome in terms of percent change in serum levels. Also, at 9 months, female rats, exposed to dietary ETU at F_0:F_1 = 30:83 and 90:83 ppm, exhibited a greater incidence of follicular cell hyperplasia (10/10) than did the female rats exposed at 0:83 (incidence = 5/10). The statistical analysis, however, used only the 0:0 groups as the control, and did not compare the 30:83 and 90:83 groups to the 0:83 group. At 2 years, male rats exposed to F_0:F_1 = 90:83 ppm exhibited a statistically significant increased incidence of follicular cell hyperplasia compared to rats exposed to F_0:F_1 = 0:83 (47/59 and 30/46, respectively). The results suggest the perinatal exposure to ETU may render some rats more sensitive to the non-cancer histopathologic changes, but sufficient data were not presented to quantify the relationship.

In a different study, rats were fed ETU in the daily diet (0, 5, 25, 125, and 500 ppm) over a period of 2-years and evaluated for non-cancer and cancer endpoints (Graham *et al.*, 1975). Weight loss (M + F) occurred at 500 ppm. Organ / body weight increases were observed at 18 months for liver at 250 ppm (M), thyroid at 500 ppm (M) and 250 ppm (F). At 24 months, organ/body weight increases were noted for heart and liver at 500 ppm (M), and thyroid at 250 ppm (F).

Graham *et al.* (1975) also measured iodide uptake into the thyroids of 5 male and 5 female rats, each at 18 and 24 months. Among the males at 18 months, statistically significant increased uptake was observed at 25 and 125 ppm and decreased uptake at 500 ppm. A non-statistically significant increase in iodide uptake was also observed at 18 months. At 24 months, iodide uptake into male thyroids increased at 5 ppm ETU and decreased at 500 ppm. Among the female rats at 18 months, increased iodide uptake was observed at 25, 125, and 250 ppm, while decreased uptake was observed at 500 ppm. Although the data are qualitatively similar to the 18 month male rat data, statistical significance could not be demonstrated. The lack of statistical significance was probably due to the large variability and the low numbers of animals. At 24 months the uptake of iodide into the female thyroid tissue was variable over the dose range. The biphasic dose-response curve obtained for iodide uptake has biological significance. Decreased iodide uptake may prevent the synthesis of adequate levels of thyroid hormones. Excessive iodide uptake, like the thiocarbamates, can inhibit the iodination of thyroglobulin, precursor to the thyroid hormones (Smith *et al.*, 1983). Because of the small numbers of animals and high variability, a NOAEL or LOAEL is not suggested for this endpoint.

Histopathology was carried out at 2 years. Increased incidences of thyroid hyperplasia and thyroid carcinoma/adenocarcinoma were observed. However, the dose-response relationships for the endpoints were different. For thyroid hyperplasia, increased incidence occurred at 5 ppm ETU, continued to increase up to 125 ppm, and then decreased at the higher ETU doses. The increased incidence of thyroid carcinoma/adenocarcinoma did not occur until 250 and 500 ppm ETU. The reverse U-shaped dose-response curve for thyroid hyperplasia resembles the curve for increased iodide uptake at 18 months. In this study, the hyperplastic response was separated from the neoplastic response based on the two dose-response relationships, wherein the latter effect was not observed until the hyperplastic response was on the decline (Graham *et al.*, 1975; U.S. EPA, 1995). The narrative does not specify which cell type in the thyroid tissue was responsible for the hyperplasia. If the hyperplasia were thyroid follicular cell hyperplasia (see the study by Chhabra *et al.*, 1992), such increased numbers of cells at the lower doses could explain the increased uptake of iodide, because iodide uptake in the thyroid occurs at the follicular cell (Smith *et al.*, 1983).

VI. Derivation of Chronic Reference Exposure Level (REL)

Study	Graham *et al.* (1975)
Study population	Charles River Rats (68 per sex per group)
Exposure method	Ingestion (0, 5, 25, 125, 250, or 500 ppm in the diet)
Critical effects	Thyroid hyperplasia
LOAEL	5 ppm in diet
NOAEL	Not observed
Exposure continuity	Daily, *ad libitum*
Exposure duration	2 years
Average experimental exposure	0.25 mg/kg-day, based on a food consumption rate of 0.05 x body weight per day (Anderson, 1983) and body weight data reported in Graham *et al.* (1975)
LOAEL uncertainty factor	3
Subchronic uncertainty factor	1
Interspecies factor	10
Intraspecies factor	10
Cumulative uncertainty factor	300
Oral reference exposure level	0.0008 mg/kg-day
Route-to-route extrapolation factor	3,500 $\mu g/m^3$ per mg/kg-day
Inhalation reference exposure level	3 $\mu g/m^3$ (0.7 ppb)

Strengths of the REL include the use of a carefully executed study using large numbers of experimental animals carried out over the lifetime of the rats. Different endpoints and dose-response relationships were evaluated. Importantly, the Graham *et al.* (1975) study included low doses (5 and 25 ppm dietary ETU). A LOAEL of 5 ppm (0.25 mg/kg-day) was reported by USEPA (IRIS, 1997) for ETU, based on the study by Graham *et al.* (1975).

The hyperplasia data from the Graham *et al.* (1975) study appears to be similar to the data of Chhabra *et al.* (1992), but differences in protocol and classification of the hyperplasia prevent a direct comparison. Whereas the 2-year incidence of thyroid hyperplasia decreased after 125 ppm ETU in the Graham *et al.* (1975) study, the 2-year incidence of follicular cell hyperplasia in the Chhabra *et al.* (1992) study increased at 250 ppm and occurred in 60-90 percent of the animals (data not shown). In the latter study, the 9-month incidence of follicular hyperplasia also increased at 250 ppm ETU. The Chhabra *et al.* (1992) study did not include adult only doses less than 83 ppm.

In the Chhabra *et al.* (1992) study, decreased T_3 and T_4 levels occurred after 9-months exposure to 83 ppm ETU, the lowest adult only dose that was used. While decreased thyroid hormone levels could be explained by decreased iodide uptake, decreased T_3/T_4 levels may also occur if excessive iodide is taken up by the follicular cells of the thyroid (Smith *et al.*, 1983). Such increased iodide uptake was observed by Graham *et al.* (1975) in rats exposed for 1.5 years to the lower ETU doses.

The use of the Graham *et al.* (1975) study to derive the inhalation exposure level is supported by data from other studies. Chhabra *et al.* (1992) observed thyroid follicular cell hyperplasia and altered thyroid functions in rats exposed to 83 ppm dietary ETU for 2 years. ETU-Hb adducts (Pastorelli *et al.*, 1985) and altered thyroid function (Smith, 1984) were observed in humans in occupational settings.

Liver changes were observed in the Graham *et al.* (1975) and Chhabra *et al.* (1992) studies. In the former study, increased liver / body weight was observed for males at 18- and 24 months at the high doses. Chhabra *et al.* (1992) recorded liver centrilobular cytomegaly at 13-weeks in rats exposed to 750 ppm ETU.

The major strength is the observation of a NOAEL for increased thyroid weight. Thyroid hyperplasia was noted at lower doses but the authors concluded that those effects were not biologically adverse.

A major uncertainty in deriving an inhalation reference exposure level (REL) for ETU is the extrapolation of exposure by ingestion to exposure by inhalation. No data were found that address the uptake and disposition of ETU by the respiratory system. A Kow = 0.22 (HSDB, 1997) might indicate a water soluble compound that would remain in the aqueous environment of the lung. The low Kow, however, does not preclude binding to specific macromolecular sites that could facilitate transport to the capillary bed that will enable delivery to the tissues. The presence of ETU-Hb adducts in the blood of ETU-manufacturing workers (Pastorelli *et al.*, 1995) and reduced thyroid hormone levels among some ETU-exposed workers (mixers) at an ETU-manufacturing plant (Smith, 1988) strongly suggest the presence of an ETU transport system across the lung. In this way, inhaled ETU may have access to the systemic circulation. Under these conditions, a route-to-route extrapolation appears justified.

Other uncertainties are the lack of adequate human exposure data, the lack of inhalation exposure studies, the lack of reproductive and developmental toxicity studies, and the lack of observation of a NOAEL.

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